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MOLDS, YEASTS, AND ACTINOMYCETES

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A Handbook for Students of Bacteriology

BY

ARTHUR T. HENRICI, M.D.

Professor of Bacteriology, University of Minnesota

WITHDRAWN

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PREFACE

The bacteriologist is continually confronted with molds, yeasts and Actinomycetes, no matter what may be the particular field of application of the science in which he is engaged. Many of these are but contaminants, as Thom says, "noxious weeds" which plague him by overgrowing his cultures of bacteria. But an ever-increasing number of fungi are found to be causes of disease processes in man and animals, while in the chemical transformations of the soil and the spoilage of foods and other organic products of industry they are of equal importance with the bacteria. The industrial uses of these microorganisms have been known to a limited extent for some time, but promise to undergo extensive development in the near future. These fungi cannot, then, be ignored by the bacteriologist.

The lower fungi are of interest, however, not alone because of their practical importance. Very significant and interesting facts are being discovered concerning their complicated life-cycles, particularly with regard to the behavior of the nuclei, and their sexual relations, which will have an important bearing upon problems of variation and evolution in this group. Since it is becoming daily more clearly evident that at least some of the bacteria belong to the fungi, and there is a well-defined movement to trace similar cycles and relationships in the bacteria, it behooves the bacteriologist to make himself acquainted with at least the essentials of this newer knowledge.

This book will, it is hoped, help fill the gap existing between the brief and inadequate discussions of the fungi found in current textbooks of bacteriology and the extensive monographs and technical articles which treat of particular groups. The latter are too extensive to be easily used, too technical to be easily understood by the student of bacteriology who generally has no foundation in mycology.

In this work I have tried to present sufficient information about fungi in general, and about those forms of importance to

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the bacteriologist in particular, so that the student will be prepared to use the more technical literature; to present such descriptions and keys that will enable him with confidence to identify some of the commoner or more important species, and at least approximately place the others within their proper family and genus; and to furnish references to general works or specific monographs where detailed information on particular forms may be found.

The book has grown out of a lecture course which has been offered for some years to advanced students in that ever-growing group that have planned their college work with the definite idea of becoming bacteriologists. It is therefore a sort of textbook. But it will also, I hope, find a field of usefulness as a handbook to be kept in the clinical or technical laboratory for reference when problems involving yeasts, molds, or Actinomycetes are encountered. I feel strongly that bacteriology has distinctly suffered from a too sharp division of the science into categories according to particular fields of application. I have therefore attempted to deal equally with the medical and industrial applications of the subject. If the book seems to be somewhat overbalanced on the medical side, this is due to the richer literature available in that field.

No claims are made for the work on the grounds of completeness or originality. It has been my task to select, condense, and where possible simplify, from the extensive available literature, such information as seemed important enough to be necessary, tangible enough to be useful, to the student of microbiology. The subject has been plagued by a tendency of its workers to create new species on the slightest provocation, to classify and reclassify, until a bewildering number of synonymous names are in use. My approach to the subject has been purely pragmatic. I have avoided discussion of debated problems of taxonomy except where such is necessary for an intelligent understanding of the literature. I have felt that to be useful the book must be kept within the scope of a small handbook, and must be made as simple as the subject matter will allow. I have therefore omitted discussions of numerous species and genera which are of no practical importance, or which are so uncommon that they are not likely to be encountered in routine bacteriological work.

The literature cited includes, for the most part, only works of a general character or specific monographs in which extensive bibliographies may be found, save that more recent contributions not found in such works have been included. I have as far as possible provided original illustrations. It has been necessary, however, to borrow illustrations of material that was not available to me. Fig. 28 was kindly supplied by the Department of Plant Pathology of this institution. Dr. H. E. Michelson of the Division of Dermatology provided the illustrations of the dermatomycoses in Figs. 63–68 inclusive. Dr. J. C. McKinley of the Division of Neurology similarly provided the drawing of Torula meningitis in Fig. 90. I am indebted to Dr. F. W. Tanner for permission to reproduce Figs. 12 and 13 from his translation of Guilliermond's "The Yeasts," and Fig. 24 from his "Bacteriology"; to Dr. P. Tate for permission to reproduce Fig. 61 from his article on "The Dermatophytes or Ringworm Fungi" in Biological Reviews; to Dr. Aldo Castellani for permission to reproduce Fig. 69 from his "Fungi and Fungus Diseases"; to Dr. W. J. MacNeal for permission to reproduce Fig. 76 from the article by MacNeal and Taylor on "Coccidioides Immitis and Coccidioidal Granuloma" in the Journal of Medical Research; and to Dr. A. H. Sanford for permission to reproduce the map in Fig. 99, from his article on "Distribution of Actinomycosis in the United States" in the Journal of the American Medical Association. Acknowledgment must also be made to Dr. Lendner for permission to translate his keys to the Mucors and Rhizopus; to Dr. Thom for permission to use his key to the groups of Aspergilli; to Dr. Castellani for permission to reproduce his table of the Moniliae; and to Dr. Waksman for permission to use his key to the soil Actinomycetes. I am indebted to my colleagues in the department of Bacteriology for many helpful suggestions, and in particular to Dr. H. O. Halvorson for his cooperation in preparing the section on alcoholic fermentation. To all of these I make grateful acknowledgment.

ARTHUR T. HENRICI.

THE UNIVERSITY OF MINNESOTA.
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MOLDS, YEAST AND ACTINOMYCETES

CHAPTER I

THE STRUCTURE AND CLASSIFICATION OF THE FUNGI

The molds, yeasts, and Actinomycetes are Fungi, a part of that subdivision of the plant kingdom known as Thallophytes. The Thallophytes are characterized by their growth in irregular plant masses not differentiated into roots, stems and leaves like higher plants. Such a mass of plant tissue is called a thallus. The Thallophytes comprise the Algae and the Fungi. The former, being provided with chlorophyll, are capable of synthesizing their food from inorganic compounds by the energy of sunlight; the latter, being devoid of chlorophyll, must depend for their food upon organic matter synthesized by other organisms, growing as either saprophytes or parasites. There is considerable evidence that the main groups of fungi have been derived from algae by a process of more or less retrograde evolution. The Lichens may be considered a third subdivision of the Thallophytes, being peculiar plants composed of algae and fungi growing together in symbiosis.

The fungi are subdivided into the true fungi, or Eumycetes, the bacteria or Schizomycetes, and the slime-molds or Myxomycetes. The relationships of the last group are not clearly understood; in fact some biologists believe that they should be included with the protozoa of the animal kingdom. The relationships of the bacteria are also in some doubt, but the Actinomycetes seem clearly to represent a transition between them and the molds. The molds and yeasts belong to the Eumycetes. In this work where the word "fungi" is used without qualification, it will refer only to the Eumycetes.

The fungi may be unicellular or multicellular. In some, as the true yeasts, the organism is always one-celled, though a few cells may be temporarily attached in irregular clusters when actively growing. Other fungi, however, may grow as one-celled organisms for a time, or in a particular environment, and become multicellular later or under changed conditions. Many of the fungi parasitic for man and animals show this dimorphism, growing in one form in the body, in another form on artificial culture media.

Mycelium.—The multicellular fungi are composed of cells arranged end-to-end to form filaments or hyphae. These filaments branch and rebranch and intertwine, sometimes even uniting or anastomosing, to form a tissue called mycelium. This mycelium may form a loose meshwork as in the molds or a compact tissue, as in the mushrooms. Thus, while fundamentally the same in their finer structure, fungi may show extreme variations in their external appearance, due simply to the degree of compactness of the mycelium.

Such variations in external appearance have to some extent been used in the past as a basis for classification, but they are not always correlated with other characters and are unreliable. The same organism may form a loose mold-like mycelium in some circumstances, and a fleshy solid tissue in others. The true relationships of the fungi are indicated by minute cell characters, and more especially by their modes of reproduction.

In some fungous tissues, however, the cells are not arranged in filaments, but form compact masses of round or polygonal cells like those of higher plants. Such a tissue is called a pseudoparenchyma; its cells have their origin in mycelium. Sometimes such masses of fleshy tissue become quite dry and firm, developing thick, hard walls; in this form they are called sclerotia, and have the property of maintaining vitality in a dormant state for long periods.

In some fungi the mycelium is not divided into individual cells by cross-walls or septa. The entire mass of mycelium forms one large cell containing many nuclei. This absence of septa makes possible a flowing of protoplasm which is quite characteristic. Such a structure (known as coenocytic) also occurs in the filaments of certain green algae, and is considered (together with other evidence) to indicate a relationship between these algae and those fungi which possess it. Consequently the latter are called Phycomycetes, or alga-like fungi. The other fungi possess a septate mycelium. In these, each cell, separated from the others by cross-walls, may contain one nucleus as in the Ascomycetes; or two as (sometimes) in the Basidiomycetes. When the latter possess two nuclei the cells show at their junctures peculiar tube-like connections called clamp connections, the significance of which will be shown later.

Thus the three main classes of fungi may be recognized, in part at least, by the character of their mycelium: the Phycomycetes possess non-septate or coenocytic mycelium (*a*, Fig. 1); the Ascomycetes, septate uninucleate mycelium (*b*, Fig. 1); and the Basidiomycetes, septate binucleate mycelium with clamp connections. These characters, however, are not the main ones upon which the classification of fungi is based, and are not absolutely constant, for the mycelium of both Ascomycetes and Basidiomycetes may be non-septate when the plant is very young, and the mycelium of Phycomycetes will develop septa in certain conditions, as for separating off spores.

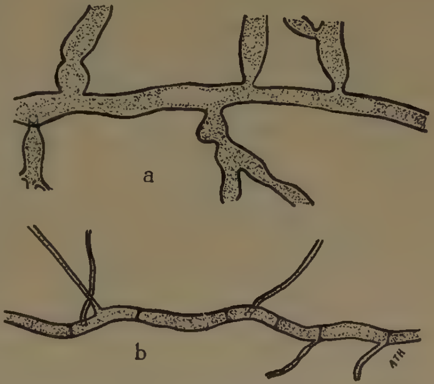

FIG. 1.—*a*. Coenocytic mycelium. *b*. Septate mycelium.

Cells of Fungi.—The cells of fungi are much like those of higher organisms in their general characters. They possess a cell wall which is frequently of appreciable thickness. This was formerly thought to be composed of a substance similar to cellulose, though it does not give the microchemical reactions of cellulose, and was called "fungus-cellulose"; but more recent investigations indicate that it may be chitin.

Within the cell wall is the cell proper, or protoplast. It contains one or more nuclei, as indicated above. These are very small and not easily demonstrated, special complicated staining methods being required. They divide by simple amitosis in the vegetative cells, by karyokinesis in some of the reproductive processes.

The cytoplasm generally presents a granular or foamy appearance due to the accumulation within it of various reserve substances in the form of granules or vacuoles. These may be of various kinds—fat, carbohydrate and protein. The amount of this reserve material varies with the age of the mycelium; in old portions of the plant it may nearly fill the cell, pushing the protoplasm to one side. Fat appears in the form of very highly refractile globules; it may be identified by staining with Sudan III. Carbohydrate is stored as glycogen, as in animals, not as starch as in green plants; it may be identified by staining with iodine. Protein is apparently stored in several forms. A noteworthy reserve substance almost peculiar to fungi is known as volutin or metachromatic material. It is identical with the material found in some bacteria designated metachromatic granules or polar bodies. It may appear either as granules or as vacuoles, the former sometimes floating in the latter. It is probably a colloid which may exist in either the sol or the gel state. There has been considerable discussion as to its true nature and function, which will be gone into in further detail in connection with the yeasts. It is generally considered a reserve material, and recent studies indicate that it is free nucleic acid. It is best identified by vital staining with neutral red.

Growth and Differentiation of Mycelium.—Although generally any part of a plant may grow and give rise to a new individual if artificially transferred to a new medium, normally fungi are reproduced by specialized cells called spores. These spores give rise to a new mycelium by germination, i.e., the protoplasm within the spore swells when in a favorable environment, bursts through the spore wall and extends outward as a long filamentous process called a germ tube. Several of these may be formed by one spore. Growth is mainly at the apex of this germ tube. At first the mycelium is non-septate, but after reaching a certain size septa are formed in those varieties which possess them, beginning in the oldest part of the mycelium and proceeding toward the periphery. Thus in a mold which is still growing there may be no septa at the tips of the filaments of mycelium. Several stages of spore germination are shown in Fig. 2.

With most multicellular fungi there can be seen a differentiation of the mycelium into two parts: a vegetative portion which burrows into the substrate, digests and absorbs it, and a reproductive portion which usually extends into the air and forms and discharges the reproductive bodies or spores. The reproductive mycelium and its spores are widely diverse in the different kinds of fungi and serve to classify and identify them. The vegetative mycelium does not vary markedly in the different groups. Not infrequently one finds molds growing on artificial culture media which do not form any reproductive bodies. They can develop only imperfectly on such a medium. Such molds are called *mycelia sterila,* and cannot be identified.

Oidia and Yeast-like Cells.—In addition to reproduction by specialized bodies or spores, many species of fungi can also reproduce by the separation of cells from any part of the mycelium, including the vegetative. Such methods, however, are not peculiar to any class of fungi and do not serve to classify. The same type may be formed by widely diverse species. But in some species they occur so regularly or are so prominent that they may be of great value in identification.

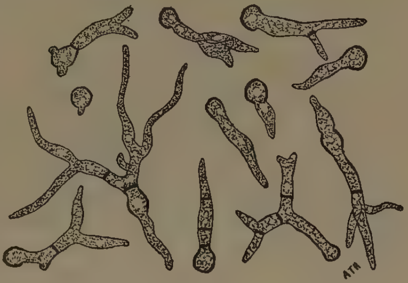

Fig. 2.—Germination of spores (conidia) of *Aspergillus glaucus.*

These free cells may be formed by a segmentation of the mycelium into its component cells by a split through the septa. The resulting cells may be cylindrical in form (Fig. 70) as in *Oidium lactis,* or may become rounded as in *Mucor racemosus* (Fig. 35). Free cells formed in this way are known as oidia. They may give rise to new free cells by division or by budding, or may give rise to mycelium. Free cells may also arise from the mycelium by either lateral or terminal budding, as also occurs with *Mucor racemosus* (Fig. 35) and with the various species of pathogenic Moniliae (Fig. 82). These may also form either new free cells (by budding) or mycelium, depending upon their environment.

Such free cells differ from spores in that they are not equipped for maintaining life in a dormant condition for periods of time. They do serve, however, to multiply rapidly and disseminate the species, especially in liquid media. They are to be looked upon as growth forms rather than specialized reproductive bodies. The conditions which determine their appearance are quite diverse for different species. Thus in Mucors they are formed only under semi-anaerobic conditions, whereas with the Moniliae they are produced to the exclusion of mycelium in aerobic cultures.

These oidia or free cells bear a great resemblance to and may be readily mistaken for true yeasts, especially when they multiply by budding. In fact, as will be shown later, yeasts are probably descended from more complexly organized fungi which have permanently lost the power to produce mycelium and maintain only the one-celled growth form. But, as was pointed out above, yeast-like cells may be formed under certain circumstances by representatives of all the great classes of fungi, Phycomycetes, Ascomycetes and Basidiomycetes. Similarly we may trace with the true yeasts, evidences (in their spores) of relationships to at least two of these classes, the Ascomycetes and Basidiomycetes. *Yeasts, then, are a heterogeneous group of fungi which maintain permanently a unicellular growth form, and are not an independent class of organisms.* Their classification together is only for convenience, and does not imply a true systematic relationship.

Chlamydospores.—With many species of fungi we may find a cell here and there in the mycelium (including the submersed or vegetative portion) which becomes differentiated from the others by increased size, due to the storage of much reserve foodstuff, and by a markedly thickened cell wall. These are known as chlamydospores. They are particularly adapted for maintaining vitality through long periods of dormancy. They remain intact and viable after the remainder of the mycelium has died and disintegrated. (See Fig. 34.)

Like the oidia, chlamydospores are not peculiar to any class of fungi, but may be formed by quite diverse species. Moreover, they are particularly likely to develop in just those species that frequently form free vegetative cells, and one may often find all transitions between the actively vegetative yeast-like cells and the dormant chlamydospores. Similarly, one may find all transitions between the vegetative oidia formed in the submersed mycelium of an organism like *Oidium lactis* and the conidia or true spores formed by its aerial mycelium. These transitions have led to much confusion in classification and nomenclature. These transitional types of growth or reproduction are particularly frequent in many of the lower forms of fungi important to the bacteriologist.

Spores.—The spores proper are very constant in their characters and mode of formation and are therefore largely relied upon for classification and identification. They may be either sexual, i.e., formed by repeated division after the fusion of nuclei from two similar or dissimilar cells; or asexual, i.e., by the division of a single cell without fusion with another. Some fungi reproduce by only one method, some by both, the latter frequently showing alternations of generation, forming non-sexual spores at one stage of their life history and sexual ones at another stage. In some parasitic fungi, the rusts, two different hosts may be necessary for the life-cycle, and several kinds of spores may be produced in succession in each host, so that the cycle becomes quite complicated.

Basidiomycetes.—The class of Basidiomycetes comprises for the most part the large, fleshy fungi, as the mushrooms, the puffballs, and the bracket fungi which grow upon trees. These are the true, or Eubasidiomycetes (Autobasidiomycetes). They reproduce by only one type of spore, the basidiospore. What we call a mushroom consists of only the spore-forming or reproductive part of the plant. There is an extensive vegetative mycelium underground.

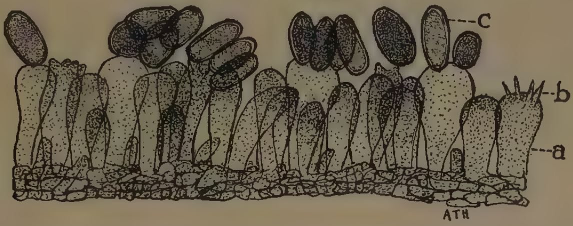

FIG. 3.—Section through the margin of a gill of a mushroom (Coprinus species) showing: *a*, the Basidia; *b*, the Sterigmata, and *c*, the Basidiospores.

Life Cycle of a Mushroom.—The stalk of a mushroom is composed of numerous filaments of mycelium arranged in a compact bundle. These terminate in the gills in the form of swollen or pear-shaped tips, the basidia. Each basidium gives rise to four basidiospores attached each to a minute stalk, the sterigma (Fig. 3).

When these basidiospores germinate they give rise to a mycelium which is uninucleate, i.e., divided by cross-walls into cells which contain but one nucleus each. If mycelium from two different spores comes in contact, the terminal cells of the two filaments in contact will fuse, and from the cell so formed there arises the extensive mycelium of the mushroom. But the nuclei of these fused cells do not immediately unite, each remaining a separate unit and dividing at each cell division (Fig. 4*a*).

One nucleus divides before the formation of a cross-wall, the other afterward; and one of the two nuclei formed by this latter division is returned to the preceding cell by a small lateral tubular process, the clamp connection. The presence of clamp connections is thus an outward sign that the mycelium is binucleate and has arisen from the fused mycelium derived from two different spores (Fig. 4, *b*, *c*, *d*, *e*).

Now when such binucleate mycelium is mature, the two nuclei in the tip cell of each filament terminating in the gills of the mushroom proceed to fuse, then divide twice, giving rise to four daughter nuclei which become the nuclei of the four basidiospores (Fig. 4, *f*, *g*, *h*, *i*). Thus the sexual act in such fungi is divided into two phases, a fusion of cells from mycelium arising from different spores early in the growth of the mushroom, and a fusion of the nuclei at a much later stage, when the plant is mature.

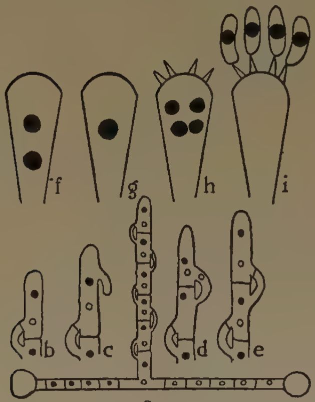

FIG. 4.—Sexual relationships in mushrooms. *a.* The uninucleate mycelium from two spores unites to form a binucleate mycelium with clamp connections. *b–e.* Stages in cell division of the binucleate mycelium showing the origin and function of the clamp connection. *f–i.* Fusion of nuclei and division in the basidium, with the formation of basidiospores.

The four basidiospores formed by each basidium may be of two sexes, i.e., the mycelium from two of them may fuse with the mycelium from the other two, but not with each other; or they may be all unlike, the mycelium from any one being capable of fusing with that from any of the others. But a spore from one basidium will be able to conjugate with only three of the four spores from another basidium, not with the fourth. Thus it is possible to have four different sexes in the basidiomycetes! These sexes are, however, not distinguishable by morphological characters.

In addition to the large, fleshy fungi, certain more minute forms are included with the Basidiomycetes because of the mode of formation of some of their spores. These are plant parasites: the smuts (Hemibasidiomycetes), and the rusts (Protobasidiomycetes).

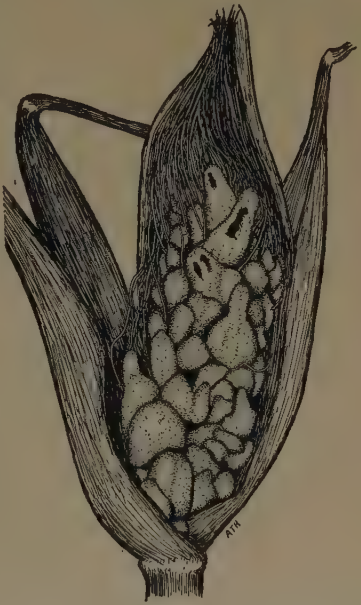

FIG. 5.—An ear of corn affected with smut.

Smuts.—As an example of the Hemibasidiomycetes we may consider the parasite of corn smut, *Ustilago zeae*. The parasite grows in the developing seeds which become markedly hypertrophied and deformed (Fig. 5). The tissue is eventually destroyed and replaced by a large mass of black spores (Fig. 6). These smut spores germinate if they lodge on a suitable substrate and give rise to a single short filament of mycelium, the promycelium.

From this promycelium there are given off a series of spindle-shaped cells, the sporidia, by lateral and terminal budding. These may remain attached to the promycelium and to each other for a time, forming a pseudomycelium, or may become detached. Detached sporidia may continue to form new cigar-shaped cells by budding, and in cultures on artificial media this type of growth predominates, only a small amount of mycelium being formed. Since smut spores are formed in enormous numbers and are widely distributed by the wind, they occasionally lodge upon cultures in the bacteriological laboratory and may frequently be found in soil cultures. In such cases they are apt to be mistaken for yeasts, since they grow mainly as single cells reproducing by budding (Fig. 7).

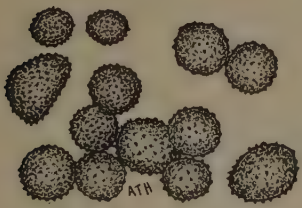

FIG. 6.—Smut spores of *Ustilago zeae*.

If the sporidia lodge upon corn, however, they give rise to an extensive mycelium which penetrates to all parts of the growing grains. After the nutrient in the grain has become exhausted, and the fungus has matured, it proceeds to form the smut spores again. These develop from all parts of the mycelium, some of the cells becoming enlarged, and enclosed in a thick black wall. During their formation these spores are surrounded by a mucoid material. The rest of the mycelium degenerates and disappears, setting free the matured spores. Because of the manner of their formation the smut spores are morphologically chlamydospores. In the parasite of corn smut these smut spores are formed by a sort of sexual process; the wall between contiguous cells is dissolved away, and their nuclei fuse. The smut spore is uninucleate, and this condition is maintained throughout the other phases of the life cycle.

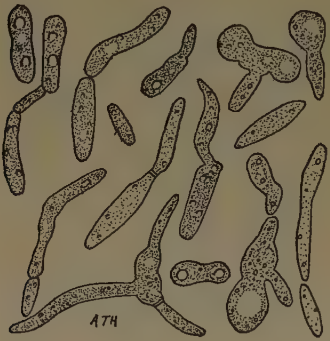

Fig. 7.—Sporidia of *Ustilago zeae* from an artificial culture.

In other species of smuts, however, the life-cycle is a little more complicated, and the relation to the other basidiomycetes more clear. The sporidia fuse two by two when in a suitable environment, but their nuclei remain separate and the fused cells give rise to a binucleate mycelium; but during the development of the smut spores, the two nuclei fuse so that the mature smut spore is uninucleate. When this germinates, the promycelium formed is sometimes typically four-celled, each of the four cells containing a single nucleus formed by the fusion nucleus of the spore having divided twice. For this reason the promycelium is considered as homologous with the basidium in the true Basidiomycetes, and the sporidia as homologous with the basidiospores. As in the higher Basidiomycetes, the sexual process is divided into two phases, a fusion of cells when the sporidia unite, with the nuclear fusion delayed until the formation of the smut spores.

Rusts.—In the rusts the cycle is still more complicated. The black stem rust of wheat (*Puccinia graminis*) may be taken as an example. It requires two hosts, the barberry and wheat, for its complete life-cycle.

The uninucleate mycelium which grows in the leaves of the barberry forms a particular kind of spore, the aeciospore, in structures called "cluster cups" on the under side of the leaf (Fig. 8). But these aeciospores are formed after a fusion of cells (without nuclear fusion) and are therefore binucleate.

The aeciospores can germinate only on wheat. They infect the leaves and give rise to a binucleate mycelium which eventually forms another kind of spore, the uredinospore. These are reddish in color and form the "red pustules" on the wheat leaves (Fig. 9). The uredinospores are also binucleate.

Toward the end of summer, as the host plant matures, the stems become infected, and from the mycelium in the stems there arises a third type of spore, the teleutospore. The teleutospores are surrounded by a thick dark-colored cell wall and are formed in the "black pustules" on the stem. They are two-celled, each spore consisting of two separate protoplasts, each in its own compartment (Fig. 10). But each cell is uninucleate, a nuclear fusion having taken place in the binucleate cells from which they were formed.

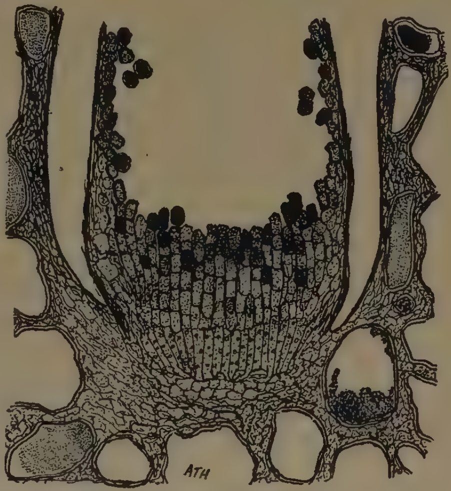

FIG. 8.—Section through a "cluster cup" on the under surface of a barberry leaf infected with *Puccinia graminis* showing the formation of aeciospores.

The teleutospores remain dormant over winter. In the spring they germinate, each of the two cells forming a promycelium. In this the nucleus divides twice, forming four, which become separated by cross-walls, so that we have a four-celled basidium, as in some of the smuts (Fig. 11). From each of these four cells a basidiospore is formed, with but one nucleus, which is capable of infecting the barberry.*

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*Still a fifth type of spore is formed on the upper surface of the barberry leaf. Its function in the life cycle is not completely understood.

Here also, then, we have the sexual process divided into two phases. Cell fusion takes place in the barberry. The aeciospores, the mycelium and uredinospores of the wheat leaf, and the mycelium of the wheat stem are binucleate. Nuclear fusion occurs in the teleutospores. These and their promycelia, the basidiospores and the mycelium in the barberry are uninucleate.

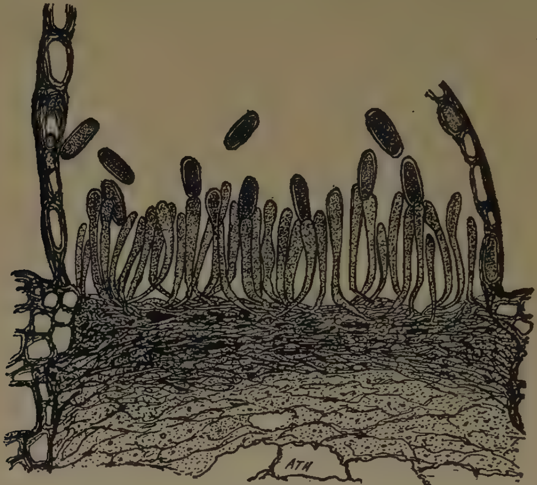


Fig. 9.—Section through a "red pustule" on wheat infected with *Puccinia graminis* showing the formation of uredinospores.

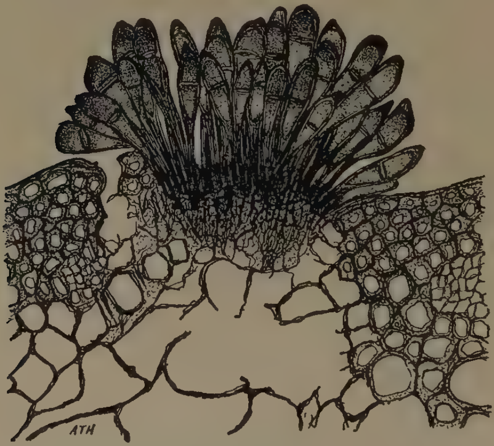


Fig. 10.—Section through a "black pustule" on wheat infected with *Puccinia graminis* showing the formation of teleutospores.

Ascomycetes.—The Ascomycetes are the largest and perhaps most important class of the fungi, including such widely different types as the large fleshy morels and the minute one-celled yeasts. Many important plant pathogens are Ascomycetes, and some of the molds important to the bacteriologist also belong to this class. It is characterized by the formation of spores called ascospores contained within a membrane or sac called an ascus. There are generally eight ascospores in an ascus, but many asci may be formed by one plant. Although there is a wide diversity in gross characters and external appearances between the various groups of Ascomycetes, the mode of formation of the ascospores is remarkably constant and indicates the homogeneity of the class.

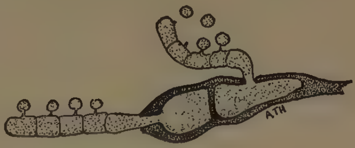


Fig. 11.—Germination of a teleutospore of *Puccinia graminis* showing the formation of promycelium and of basidiospores.

Ascospores.—The ascospores are sexual spores. The mechanism of their production is simplest in the yeasts. Different stages in the formation of the ascospores of a yeast, Schizosaccharomyces, are shown in Fig. 12. Two contiguous yeast cells send out minute tube-like processes which meet and fuse; their nuclei come together and unite, the single nucleus resulting undergoes division three times to form eight daughter nuclei. Each of these becomes surrounded by a certain amount of reserve material, and a spore wall is formed. The cell containing these eight spores is the ascus.

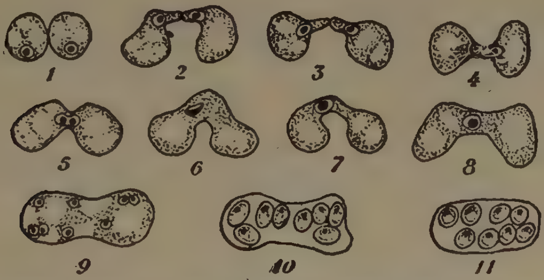


Fig. 12.—Various stages in the formation of ascospores in a yeast, *Schizosaccharomyces octosporus*. After Guilliermond.

In the genus Endomyces, a group of molds closely related to the yeasts, the formation of ascospores is almost equally simple. Two contiguous cells in a filament of mycelium send out bud-like processes. Sometimes one of these is larger than the other, i.e., there may be a differentiation into an antheridium and oögonium.

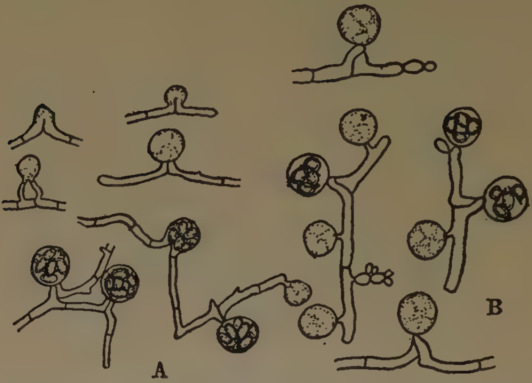


Fig. 13.—Various stages in the development of scospores in *A*, *Eremascus fertilis*, and *B*, *Endomyces fibuliger*. After Guilliermond.

These unite, their nuclei fuse, then proceed to form spores as in the yeasts. Various stages are shown in Fig. 13.

In most of the Ascomycetes, however, the process is not so simple. After fertilization of the oögonium by the antheridium, the resulting cell does not at once develop ascospores, but instead gives rise to new filaments of mycelium, the ascogenous hyphae. The next to the last cell in these hyphae is binucleate. These binucleate cells develop into asci, their nuclei fusing and then dividing. Thus there are two nuclear fusions concerned in the production of ascospores in these higher forms.

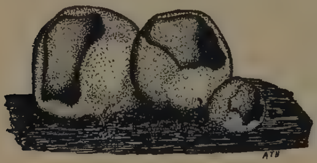


Fig. 14.—Apothecia of the cup fungi (Peziza) growing on rotting wood.

Moreover, whereas in the lower ascomycetes the ascospores may be formed on any part of the mycelium, in the higher forms they are developed in particular areas and become surrounded and protected by a dense mass of tissue, thus forming a special fruiting organ. This may be arranged as a hollow sphere (the perithecium) or as a cuplike structure (the apothecium). The fruiting bodies of the cup fungi (Fig. 14) are apothecia. Their inner walls are lined by the asci which appear as in section in Fig. 15.

Conidia.—In addition to the sexual ascospores, the Ascomycetes also form non-sexual spores called conidia. These are generally borne free at the ends of filaments of aerial mycelium, the conidiophores. In many species, ascospores are formed only rarely or in a particular environment. With most varieties grown in the laboratory on artificial culture media, ascospores are practically never seen.

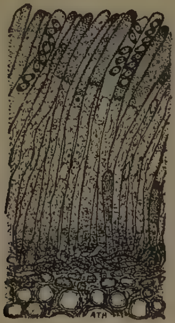


Fig. 15.—Section through an apothecium of Peziza showing asci. Some are undeveloped. Two contain the full complement of eight ascospores.

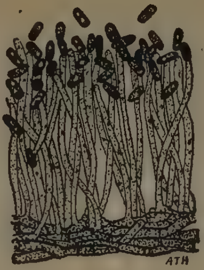


Fig. 16.—Formation of conidia by *Claviceps purpurea.* Stained section of a grain affected with ergot. Only a portion of the fungus on the surface is shown.

Life Cycle of Ergot.—The parasite (*Claviceps purpurea*) producing a disease of grains known as ergot may be taken as an example to illustrate the life history of a typical ascomycete. This organism is of some importance in medicine, since it produces in the infected grains a substance which produces contractions of involuntary muscles. It has produced poisoning both in man and domestic animals, producing abortion by inducing contractions of the uterus, and gangrene by causing constriction of blood vessels. It is also used as a drug to produce a firm contraction of the uterus after childbirth.

The fungus grows in the seeds, which, as they develop, become much larger than the healthy grains. The tissue of the grain is gradually replaced by mycelium. During this period the fungus reproduces by the non-sexual conidia (Fig. 16) formed on the surface of the grain. These are accompanied by a secretion of "honey-dew" which attracts insects. These carry the conidia to the flowers of other plants, which thus become infected.

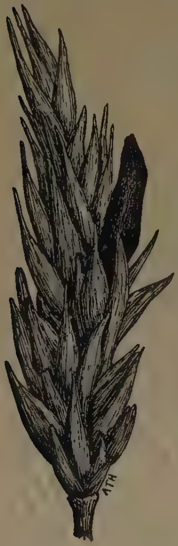


Fig. 17.—A head of grain affected with ergot showing a sclerotium.

As the grain ripens, those seeds which are infected are completely replaced by the mycelium, which now dries out and forms a dense, hard, black mass, the sclerotium. This retains the general form of the seed but is larger and projects considerably from the head of grain (Fig. 17). It is these "ergot grains" which contain the poison.

They fall to the ground and remain dormant over winter. In the spring, they revive and sprout a number of little stalks which develop rounded masses of tissue at their tips (Fig. 18). These are called stromata. In each stroma a number of pear-shaped spaces, the perithecia, are formed (Fig. 19). One of these is shown in section in Fig. 20. Each perithecium contains a number of asci, and each ascus contains eight needle-like ascospores. When mature, they are forcibly discharged into the air, and carried by the wind. If they lodge on grain, they germinate, forming a mycelium which invades the growing grain and starts the cycle over again.

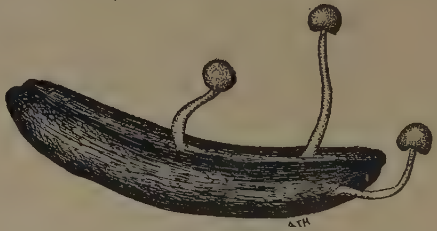


Fig. 18.—"Germination" of a sclerotium of *Claviceps purpurea*, showing the formation of stromata.

Subclasses of Ascomycetes. — The Ascomycetes are subdivided into three main divisions or subclasses, according to the mode of formation of their ascospores, or rather the way in which they are borne upon the plant. The first, the Plectomycetes, contains the more primitive forms in which the asci are formed by any of the cells (as in the yeasts) or on any part of the mycelium (as in

Endomyces); or, if they are formed on a specialized part of the plant, they do not possess a protecting envelope of mycelium (the ascocarp) or at the most a primitive and irregular ascocarp. The second subdivision contains those varieties in which the asci are borne on cup- or saucer-like apothecia; they are called the Discomycetes. The third group, the Pyrenomycetes, comprises those forms whose asci are contained in perithecia.

Fungi Imperfecti.—There are many fungi which possess the characteristic mycelium of Ascomycetes, which reproduce by conidia similar to those formed by known Ascomycetes, yet which do not form ascospores, or whose ascospores have not yet been discovered. These are designated imperfect fungi, or Fungi Imperfecti. It must be confessed that probably in many cases the imperfection lies in our knowledge of the organism rather than in the organism itself. They must be classified and identified by their conidia. The Fungi Imperfecti are recognized as a class having an equal value with the Basidiomycetes, Ascomycetes, and Phycomycetes, though this arrangement is admittedly an artificial one for convenience only. A large proportion of the fungi which we shall consider belong to the class of Fungi Imperfecti. From time to time the perfect forms of these are discovered and then the species are removed from the class of Fungi Imperfecti and placed in their proper place in the Ascomycetes. This leads to some confusion. Thus the large genus Aspergillus is placed in the Fungi Imperfecti because most of the known species do not form ascospores; but such sexual spores have been found in some species, which are therefore included by some authors in another genus, Eurotium, of the Ascomycetes. It might be argued that finding

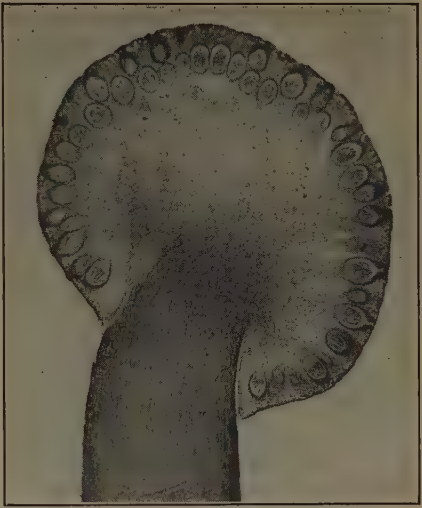


FIG. 19.—Stained section of a stroma of *Claviceps purpurea,* showing numerous perithecia at the periphery.

ascospores in one species of a genus of imperfect fungi would warrant transferring the whole genus to the Ascomycetes. But this cannot be safely done, since the genera of Fungi Imperfecti are based upon the mode of formation of their conidia; and it is known that widely diverse types of Ascomycetes may produce the same sorts of conidia.

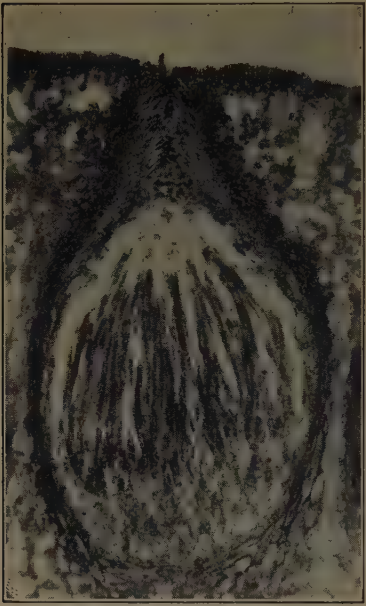


FIG. 20.—Stained section through a perithecium of *Claviceps purpurea* showing the elongated asci. Each of these contains eight needle-like ascospores.

Phycomycetes.—The Phycomycetes are the most primitive class of the fungi. They reproduce both by sexual spores and non-sexual spores. There are three subclasses which are so diverse that the class can best be described by considering each subclass separately.

The Archimycetes are primitive forms, mainly aquatic and parasitic on water plants and animals, though some are important causes of disease of land plants. They are characterized by the absence of mycelium, the plant being a single cell or an irregular mass, though a rudimentary mycelium is formed by some. They reproduce mainly by motile, flagellated spores called zoöspores. In some, sexuality is very primitive. In others, well-defined gametes, an oögonium and antheridium, are formed. There are no forms of importance to the bacteriologist.

The Oömycetes are also mostly aquatic forms, some are parasitic on land plants, and some live in soil. They reproduce by sexual spores called oöspores, and non-sexual spores which in many forms are also motile zoöspores.

Life Cycle of Saprolegnia.—The common water molds of the genus Saprolegnia may be considered to illustrate the life history of an Oömycete. They are found especially on dead animal matter submerged in water, as insects, fish, etc. Some are parasitic on fish. The scum which develops frequently on goldfish in aquaria or minnows kept in bait pails is due to the growth of this mold. They are especially prone to develop on fish after the latter have been handled or bruised (Fig. 21).

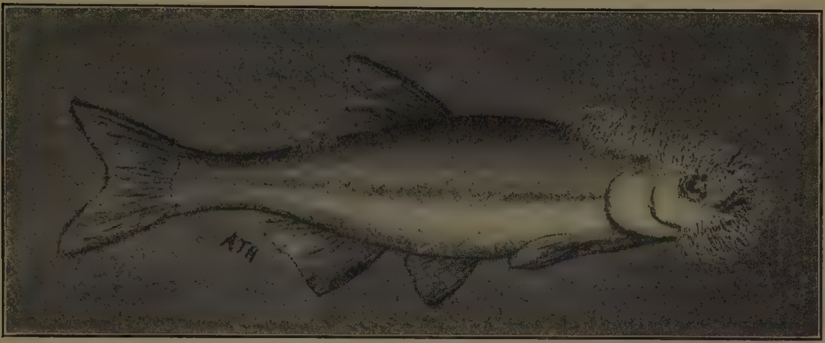


Fig. 21.—A minnow infected with Saprolegnia.

The non-sexual spores are formed at the tips of filaments of mycelium projecting into the water. The terminal portion of the filament becomes separated from the rest by a cross-wall. The cell so cut off contains many nuclei. Vacuoles appear in various parts of this cell and by growing and coalescing gradually cut up the enclosed protoplasm into a number of small cells, which develop a cell wall and finally two flagella. They are the zoöspores, and the cell in which they are formed is a zoösporangium (Fig. 22).

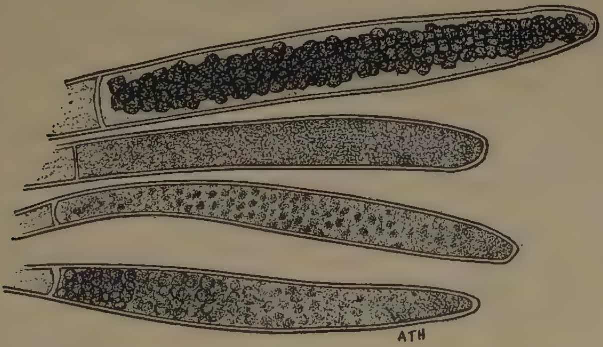


Fig. 22.—Sporangia of Saprolegnia with zoöspores in various stages of development.

The zoöspores are uninucleate. When mature the zoösporangium opens at the tip and the motile zoöspores swim away to seek a new substrate. When such is found, they lose their flagella and germinate by putting forth filaments of mycelium.

Sexual spores are not formed so frequently. They are produced by the conjugation of two morphologically unlike cells, a large oögonium and one or more small tube-like antheridia. These generally develop from neighboring branches of the same filament. Both the oögonium and the antheridium are at first multinucleate like the coenocytic mycelium from which they are derived. They become separated from this mycelium by cross-walls. The protoplasm of the oögenium becomes divided into cells containing each a single nucleus. The antheridium penetrates the oögonium and its nuclei fuse with those of the cells of the oögonium, each cell receiving one nucleus from the antheridium. These fertilized cells then become the oöspores, by enlarging and developing a thick membrane. They are capable of remaining dormant for considerable periods of time.

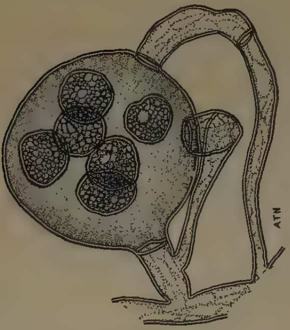


Fig. 23.—An oögonium of Saprolegnia. It has been fertilized by two antheridia and contains six oöspores.

Thus in Saprolegnia there are two types of spores formed, the motile non-sexual zoöspores formed in zoösporangia, and the non-motile sexual oöspores, which are primarily resting spores, formed by the fusion of unlike gametes. In some of the terrestrial forms of Oömycetes parasitic on plants the non-sexual spores are conidia, as in the Ascomycetes and Fungi Imperfecti, formed by abstriction of cells at the tips of the aerial filaments.

Life Cycle of Mucor.—In a typical example of the Zygomycetes, the third subclass of Phycomycetes, the multinucleate character is maintained throughout the life-cycle. Mucor may be taken as an example. The aerial mycelium gives rise to non-sexual spores in a sac or membrane, the sporangium. The terminal portion of the filament becomes greatly enlarged, vacuoles appear within the protoplasm, and by enlarging and coalescing these gradually separate the protoplasm into individual cells. The spores so formed contain each several nuclei. They mature by developing a spore wall. They are known as sporangiospores.

The filament which bears the sporangium is called a sporangiophore. Its inflated tip, which projects into the sporangium, is the columella. When the spores are mature the sporangium membrane dissolves away, liberating the spores, which are distributed by the wind. A portion of the sporangial wall remains attached to the base of the columella. (See Fig. 33.)

The sexual spores of the Zygomycetes are named zygospores, and are formed by the fusion of elements which are morphologically alike, i.e., they are not differentiated by size into an oögonium and an antheridium, as in the Oömycetes. They may be formed by the fusion of two hyphae from the same plant, in which case the species is said to be homothallous; or only by the fusion of filaments from separate plants, in which case the species is heterothallous.

When two hyphae capable of conjugating come together, their terminal portions become separated by cross-walls. Their cell walls dissolve away where they touch, and the two multinucleated masses of protoplasm run together, the nuclei proceeding to unite two by two. The cell develops a thick wall with generally warty or spiny projections from its surface. Various stages in the formation of a zygospore are illustrated in Fig. 24.

Zygospores, like oöspores, generally remain dormant for considerable periods. When they revive, they usually germinate by putting forth a single filament which at once forms a sporangium and sporangiospores.

While there is no morphologically distinguishable differentiation into two sexes in the Zygomycetes, it can be shown with the heterothallous species that there is a physiological differentiation. By inoculating spores from different plants on the two halves of a Petri plate culture, it will be found that in some cases where the two plants come together the filaments will fail to fuse and form zygospores, whereas with other combinations they will. And by repeating this experiment with many strains one can demonstrate that there really exist two sexes, though they cannot be distinguished by their appearance. These "sexes" are referred to as plus and minus strains.

In the Zygomycetes then we also have two types of reproductive bodies, the non-sexual sporangiospores, and the sexual zygospores. The main difference between the Zygomycetes and the Oömycetes lies in the mode of formation of the sexual spores. The zygospores of the former are produced by the union of morphologically alike elements (isogamy); the oöspores of the latter result from a fusion of morphologically differentiated elements (heterogamy).

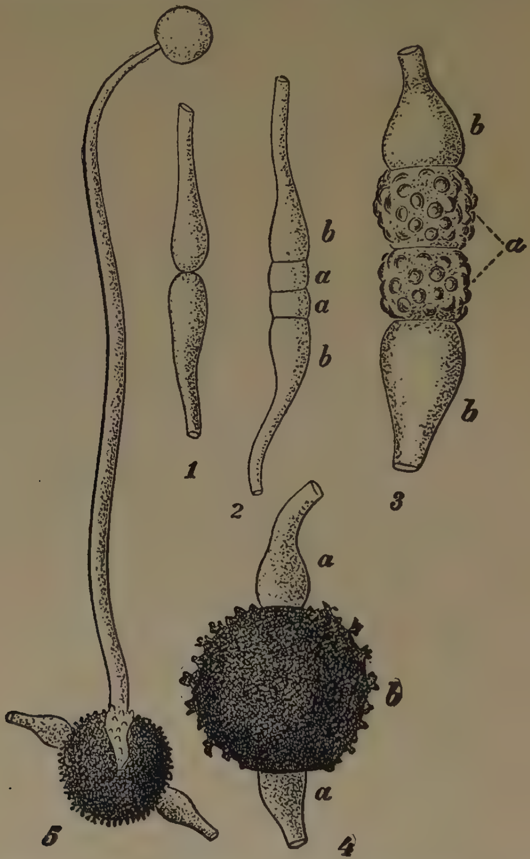


Fig. 24.—*Mucor mucedo.* Formation of the Zygospores. (After Brefeld.) 1. Two hyphae in terminal contact. 2. Articulation into gamete *a* and suspensor *b*. 3. Fusion of the gametes *a;* the membrane thickens. 4. Ripe zygospore *b* supported by the suspensors *aa*. 5. Germination of the zygospore to a sporangium stem.

Origin and Evolution of the Fungi.—In many respects the Phycomycetes resemble very closely certain of the green algae, or

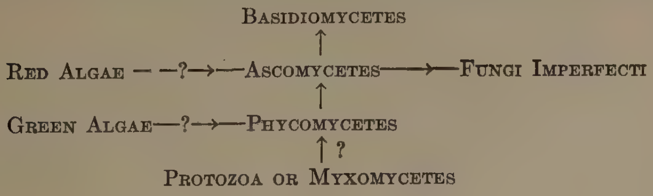
Chlorophyceae. Particularly the aquatic forms like Saprolegnia are very much like algae of the types like Vaucheria, differing mainly in the absence of chlorophyll and consequently the mode of nutrition. Reference has already been made to the multinucleate character of the protoplasm common to these two groups. In addition, both the production of non-sexual zoöspores in a sporangium, and of sexual oöspores by the fertilization of an oögonium by an antheridium are almost identical in the two cases. It is therefore highly probable that the Phycomycetes have had their origin from the green algae. Having adopted a parasitic or saprophytic habit of life they have lost chlorophyll, and have undergone further evolution as a result of having become terrestrial.

However, the more primitive types of Phycomycetes, the Archimycetes, show some resemblance to certain of the slime molds (Myxomycetes) and also to some of the Protozoa, and may have developed from some such simple form. There are therefore two distinct views regarding the origin and evolution of the Phycomycetes.

Also there is some difference of opinion concerning the origin of the Ascomycetes. The process by which the ascospores are formed is closely paralleled by the sexual reproduction of the red algae or Rhodophyceae. Previously they were considered to have been derived from these seaweeds, as the Phycomycetes were believed to have developed from the green algae. More recently, however, there have been found many transitions between some of the Phycomycetes and some of the simpler types of Ascomycetes, and it is quite possible that the latter have had their origin in the former.

The Basidiomycetes have no parallel in the algae. On the other hand, it is fairly clear that they represent a further evolution of the Ascomycetes.

The following diagram may serve to make more clear the various theories concerning the origin and evolution of the fungi.



A KEY TO THE CLASSES AND SUBCLASSES OF FUNGI

Mycelium usually non-septate (coenocytic).	*PHYCOMYCETES.*
Mycelium septate:	
Sexual spores exogenous, borne on stalks	*BASIDIOMYCETES.*
Sexual spores endogenous, in a sac.	*ASCOMYCETES.*
No sexual spores.	*FUNGI IMPERFECTI.*

Subclasses of Phycomycetes

Mycelium absent or rudimentary.	*ARCHIMYCETES.*
Mycelium well developed:	
Sexual reproduction heterogamous; non-sexual spores motile.	*OÖMYCETES.*
Sexual reproduction isogamous; non-sexual spores non-motile.	*ZYGOMYCETES.*

Subclasses of Ascomycetes

Asci irregularly arranged with no protective tissue, or if such is present, irregular or without a definite opening.	*PLECTOMYCETES.*
Asci borne in regular fruiting organs:	
Fruiting organs are apothecia.	*DISCOMYCETES.*
Fruiting organs are perithecia.	*PYRENOMYCETES.*

Subclasses of Basidiomycetes

Number of basidiospores indefinite.	*HEMIBASIDIOMYCETES.*
Number of basidiospores definite, usually four:	
Basidium septate	*PROTOBASIDIOMYCETES.*
Basidium non-septate, continuous	*AUTOBASIDIOMYCETES.* (Eubasidiomycetes).

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CHAPTER II

METHODS FOR STUDYING MOLDS, YEASTS AND ACTINOMYCETES

Isolation of Molds.—Molds may be cultivated on either solid or liquid media in tubes or dishes, as are bacteria. But molds generally grow more slowly than bacteria, and where both are present in the material to be examined, the latter are apt to overgrow Petri plate cultures before the former can develop. For isolation it is therefore desirable to use a medium favorable to the growth of molds and unfavorable for bacteria. Such a medium may be obtained by adding to ordinary nutrient agar a rather large amount of sugar, and by making the medium rather strongly acid, since most molds will tolerate higher degrees of acidity than will bacteria. A medium containing 5 per cent dextrose and 0.5 per cent tartaric acid in addition to the usual meat extract and peptone is very satisfactory.

If agar is autoclaved in such an acid medium, however, it will be markedly hydrolyzed, and will fail to jell on cooling. This difficulty is obviated by sterilizing the dextrose and acid separately in concentrated solution. When highly concentrated, the dextrose is not so markedly changed in sterilizing. A solution containing 50 per cent dextrose and 5 per cent tartaric acid may be safely autoclaved. Such a sterile solution may be kept on hand, and when need for a medium for isolating molds arises, it can be quickly met by melting a deep tube (about 10 cc.) of ordinary agar and adding to it 1 cc. of the dextrose-tartaric solution. This gives a final concentration of about 5 per cent dextrose and 0.5 per cent tartaric acid, and a reaction of about *p*H 3.8. On this medium most molds and yeasts will grow luxuriantly, most bacteria not at all.

The growth on the above medium is for some purposes, as in counting molds in soil, for instance, almost too luxuriant, since some of the rapid growers tend to spread over the plate and crowd out others. Where a number of molds are to be expected, therefore, a medium somewhat less rich in nutrient may be preferred. Such a medium has been devised by Waksman[12] especially for soil work, but will prove of value for isolations from other material. It consists of

Dextrose	10.0 gm.
Peptone	5.0
Monobasic potassium phosphate	1.0
Magnesium sulphate (crystals)	0.5
Agar	15.0
Water	1000.0 cc.

Just before use, i.e., after sterilizing and while still melted, add half normal sulphuric acid until the reaction is *p*H 3.8 to 4.0. This medium has much less nutrient than the preceding. By using a mineral acid there is no danger of the acid's being destroyed by the growth of the mold, as might occur with tartaric acid, thus eventually allowing the bacteria to develop.

Although these acid media serve well for the isolation of most of the common molds and yeasts, they will not permit a growth of all fungi. Many of the pathogenic species in particular may fail to grow on these media. By use of the dextrose-tartaric acid agar the author has succeeded in isolating the organism of sporotrichosis in one case where it was associated with numerous bacteria; in several other cases, however, this parasite failed to grow on the acid agar, whereas a good growth was obtained on dextrose-agar without acid. In such cases, therefore, it is best to make cultures on two sets of plates, one with and one without the acid. The Actinomycetes prefer an alkaline medium and will not grow at all on the dextrose-tartaric agar.

Although for most purposes isolation by plating is readily accomplished, occasions may arise when other procedures are to be preferred. Where several molds are closely crowded together on a plate, it is sometimes quite feasible to pick off a spore-head of a desired species with a sterilized wire under the higher-powered lenses of a binocular dissecting microscope. For certain types of work, as investigations of apparent mutation, cultures known to have developed from a single spore are necessary. Single spores may, of course, be picked up by the use of a micromanipulator (such as the Chambers instrument) more readily than bacteria, but much simpler procedures are available. Sass[9] has described such a method which he has used for growing single-spore strains of mushrooms. A spore suspension of proper density is sprayed over the surface of a sterile agar plate, which is then incubated for a time. The plate is then examined for well-isolated spores which have germinated, using the low-power microscope lens. When such a spore is found, a small sterilized spatula with a minute hole in it is placed over the spore and pressed into the agar in such a way as to force a small cylinder of agar (bearing the spore on its upper surface) through the hole. The growing spore may then be easily removed with a sterile needle without danger of touching any other spore.

Cultivation of Molds.—After pure cultures have been obtained, most of the fungi will grow fairly readily on all of the ordinary bacteriological media, but usually grow better if sugar is added. A number of organic substrates are recommended, but save in exceptional cases they have no great advantage over media which are always available in bacteriological laboratories. Moistened slices of bread, slices of potato or carrot, are used, as well as decoctions made from horse manure, potatoes, beans, prunes or raisins. These latter may be made solid by the addition of agar. Some of the fungi will produce fruiting bodies on these complex media which will not mature on the more simple media of the laboratory. Beer wort and wine must are especially frequently used abroad, particularly for the cultivation of yeasts.

For merely obtaining material for observation, and for maintaining stock cultures, Sabouraud's medium is most widely used, particularly by those dealing with animal pathogens. It contains as nutrients 1 per cent of peptone and 4 per cent crude maltose. This medium is especially useful for isolating and identifying fungi from the various skin diseases caused by fungi. Since the cultural characters of fungi may vary considerably with slight changes in the composition of the medium, it is insisted that for the identification of the Trichophytons and similar fungi the medium must be made from the same ingredients as used by Sabouraud. These are the "crude maltose of Chanut" and the "granulated peptone of Chassaing" and are to be obtained from the Maison Cogit, 36 Boulevard St. Michel, Paris. Such a medium is known as Sabouraud's "proof agar" (milieu d'epreuve). But for ordinary work, any peptone or maltose will serve. The maltose may well be replaced by dextrose. In this laboratory we use 1 per cent of

Difco peptone and 5 per cent crude dextrose. It is solidified by adding 1.5 per cent agar. Crude dextrose generally gives a better growth than the purified sugar. Corn syrup may be used. For most purposes it is not spoiled by sterilizing in the autoclave. On it practically all molds, yeasts and Actinomycetes will yield a rapid and abundant growth.

For the determination of enzyme action the usual bacteriological media, such as gelatine, litmus milk, coagulated blood serum, and sugar broths with gas traps and indicators may be used. The concentration of sugars should be greater than that used for bacteria; 2.5 per cent is best. In addition to these it is frequently desirable to determine the action of fungi on starch and cellulose. The former may be determined by growing the organism in Petri dishes on agar to which a little sterilized starch paste has been added. After growth has taken place the agar is flooded with Gram's iodine solution, which will stain the starch blue but will leave a clear zone about the mold if it has a diastatic action. The action on cellulose may be observed by growing in a medium containing various nutrient salts, an inorganic source of nitrogen, but no source of carbon save strips of filter paper. If the mold can digest cellulose, the paper will be softened and eventually dissolved.[3]

Many of the molds will grow on media of very simple composition if an organic source of carbon is provided, and there have been devised a number of so-called synthetic media made up largely of inorganic salts. Some of these, as Raulin's solution, which is extensively used in France, are unnecessarily complicated, containing a number of ingredients which are not at all essential. Probably the one of greatest usefulness is Czapek's medium, as modified by Dox and by Thom:[10]

Sucrose	30.0 gm.
Sodium nitrate	2.0
Dibasic potassium phosphate	1.0
Magnesium sulphate (crystals)	0.5
Potassium chloride	0.5
Ferrous sulphate	0.01
Water	1000.0 cc.

This may be solidified by the addition of 15 gm. of agar. Most of the fungi will grow fairly well on this medium, as will also the

Actinomycetes. Since, as was pointed out above, the characters of fungi may vary considerably with changes in the composition of the medium used for their identification, it would be highly desirable to have a medium of standard composition which could be prepared from pure chemicals and so would be identical in different laboratories. Pigment production is particularly variable with different media. The above-named Czapek's agar comes the nearest to such a standard agar. It has been used by Thom for descriptions of types of Penicillium and Aspergillus and by Waksman for descriptions of Actinomycetes, where color is of great importance in identification. It should be noted that Mucors cannot readily utilize sucrose and will therefore not grow well on Czapek's agar.

With a "synthetic" medium such as those described, one may readily vary the sources of both nitrogen and carbon and thus determine the ability of the organism to utilize a variety of foodstuffs. A number of such physiological studies of nutrition of fungi are available in the literature.

Determination of Morphology.—The identification of fungi depends more upon morphological characters than is the case with bacteria. For observing the morphology of molds a plate culture is much to be preferred to one in a test-tube. The naked eye appearance of the plant mass is frequently quite characteristic, and in many cases the organism can be recognized at a glance. It should be borne in mind, however, that both gross morphology and microscopic characters may in some cases vary not only with changes in the composition of the medium but also with the age of the culture.

The first step in identifying a mold is to determine whether it belongs to the Phycomycetes or the Ascomycetes (or Fungi Imperfecti). In general (though there are exceptions) this may be readily done by a rather superficial examination. With those species which belong to the Fungi Imperfecti (or Ascomycetes) the plant mass is usually relatively compact, the aerial filaments of mycelium are relatively short, and the surface is thickly covered with spores which are frequently brightly colored. The surface may be compared to the nap of velvet. With the Phycomycetous molds the mycelium is coarser and looser in texture, the aerial hyphae are longer, and the sporangia are less numerous. The spore heads and frequently the aerial mycelium are usually dark colored, brown, gray or black. The whole plant mass has a texture comparable to cotton wool.

Molds take up considerable water from the medium and give off considerable into the air, which may condense in droplets on the surface of the plant. Large amounts of this transpiration water are characteristic of certain species. The droplets are frequently colored by pigments excreted by the mold. Beginners are frequently apt to mistake these droplets of moisture on the mycelium, especially if it is colored, for spores or other structures.

The general characters of the aerial mycelium and the spore-heads may be determined by examining the growth with a magnifying glass or the low-power lens of the microscope from above, after removing the cover of the dish. For this purpose a binocular dissecting microscope is ideal.

After examining the upper surface of a Petri plate culture, one should reverse the plate and note the under surface of the "colony." Frequently very characteristic colors are produced in the submersed mycelium or in the medium itself. These will vary markedly with the medium. Some of the pigments act as indicators, being perhaps yellow on one side of neutrality and red on the other. Occasionally one may find pigment only at the spot where two different molds come together. Particularly one should note if the submersed mycelium is light in color or dark. This is important as a primary separation of molds belonging to the Fungi Imperfecti.

Microscopic Examination.—For finer details it is necessary to prepare slides. This may be done by removing a portion of the mold with a needle or pair of fine forceps and teasing it out with a pair of needles in a drop of water, then covering this with a coverglass. One may thus determine with certainty the presence or absence of septa in the mycelium, the structure of the sporophores and spores, the presence or absence of chlamydospores, etc. Such a preparation, however, shows all the parts disarranged and is not permanent. Permanent preparations showing the complete structure of the plant may be easily made with most molds by growing them directly on slides or coverglasses.

Slide Cultures.—Slide cultures may be made either with solid or liquid media, but for most purposes agar is much to be preferred. It is best to have the medium not too rich in nutrients, since then the mycelium becomes rather densely packed and it is difficult to make out details of structure. A medium containing about 2 per cent of dextrose and 0.4 per cent of peptone will prove suitable for most species. The agar should be as clear as possible, since the presence of precipitates obscures the field. Since but small amounts are required, it is quite practical to filter the agar through paper. The slides or coverglasses used must be thoroughly clean and free from grease, so that the medium will spread in a thin, uniform film. They may be kept in the usual acetic acid-alcohol mixture until used.

Coverglasses are to be preferred, since they require less equipment for incubation than slides, a number being readily incubated in a Petri dish. Place a piece of blotting paper or several thicknesses of filter paper or paper toweling in the bottom of a Petri dish, and sterilize in the hot-air oven. Just before using, wet the paper with about 5 cc. of sterile water. Clean the coverglasses, sterilize by flaming, and place them on the surface of the paper. Melt a tube of the agar in a waterbath, cool to between 45° and 50° C., and inoculate rather heavily with spores of the mold to be studied. With a wire loop deposit one or two loopfuls of the agar on each coverglass and spread in a rather thin film. The Petri dish may then be incubated. If sealed in a can, evaporation will be retarded. It is essential to maintain an atmosphere saturated with moisture.

After the molds have grown sufficiently, they may be examined directly, mounting the coverglass on a drop of water, or they may be made into permanent preparations. In the latter case, several procedures are available, depending upon the purpose of the slide. For general morphology of the fungus, the following procedure presents the advantages of simplicity and clearness of the slide.

Dry the agar film, then mount the coverglass on a drop of Amann's medium, which has the following composition:

Phenol, crystals	20 gm.
Lactic acid, syrup	20
Glycerol	40
Water	20

These are dissolved together with gentle warming, then add

Cotton blue	0.05 gm.

This is the formula as given by Linder[5] save that the amount of dye is greatly reduced. It serves as a combined fixing agent, stain and mounting fluid. The mycelium and spores will be stained blue, removing much of the dye from the solution.

Enough fluid must be used to fill the space under the coverglass without air bubbles. The latter may be largely avoided if the aerial mycelium is wetted down with a very small drop of 70 per cent alcohol just before mounting. The excess fluid is removed from about the edge of the coverglass and the slide may then be sealed. Various cements are recommended for this purpose, balsam and gold size being most widely used. An especially safe procedure is that proposed by Diehl.[1] The coverglass is first mounted in the mounting fluid on a larger coverglass. The latter is then inverted on a drop of balsam on the slide. In any case it is advisable to let the slide stand for a few days before sealing in order to let the fluid evaporate at the edge of the coverglass. Otherwise there is a tendency for the cement to run under the coverslip by capillary action. I have recently found that clear "Duco" makes an excellent cement. It dries rapidly, shows little tendency to run under the coverglass, and does little harm if it does, as it seems to mix with the mounting fluid to a certain extent. I do not know yet how permanent such mounts will be.

Slides prepared as above show very little shrinkage or distortion of the mycelium. On the other hand, they reveal but little of its internal structure. Stained mounts may be prepared and permanently mounted in balsam. They will usually be much shrunken, but for some purposes will be superior to glycerine mounts. The agar film should first be dried to avoid washing it off in subsequent handling. It may be fixed with any of the usual fixing agents, depending on the stain to be used. For ordinary purposes the formalin-alcohol-acetic mixture commonly used for plant material is satisfactory. It is made of

Alcohol (50 per cent)	100 cc.
Formalin (40 per cent)	6.5 cc.
Acetic acid (glacial)	2.5 cc.

Immersion for a few minutes in this solution will suffice.

The stain must be chosen with a view to its effect on the agar. Many stains cannot be used because they will stain the agar more deeply than the fungus. For routine slides, I have not found anything that works better than the acid thionin solution introduced by Frost[4] for staining microcolonies of bacteria. The formula is:

Thionin	1.0 gm.
Phenol	2.5 gm.
Acetic acid (glacial)	20.0 cc.
Water	400.0 cc.

About one minute is sufficient for staining. If prolonged, the agar will stain too deeply. The slides or coverglasses may then be thoroughly dried and mounted in balsam. For fine details Heidenhain's haematoxyline method may be used. The agar will stain, but will decolorize more rapidly than the fungus in the iron alum solution.

The difficulties encountered with the staining of the agar may be obviated by growing the organisms in drops of liquid medium on the coverglasses, but in this case extreme care must be taken to avoid washing off the organisms after they have been fixed. It is best to mount the coverglass in a drop of water on a slide and apply the various reagents to one side with fine pipettes, removing them from the other with blotting paper.

Another type of slide culture will prove of great value, particularly in determining details of the arrangement of the aerial mycelium, for example in untangling the branching of the sporangiophores of Mucors. Rather large coverglasses (24 × 40 mm.) are cleaned. With a small hot iron (for instance, the heated end of a small file) a drop of sealing wax, or better, de Khotinsky cement, is deposited on each end. With the hot iron this is then spread out to form a layer about 5 mm. wide and perhaps a millimeter thick across the ends of the coverglass. A clean slide is now heated in the bunsen flame and the coverglass placed upon it with the cement side down. The slide should be just hot enough to soften the cement so that it will adhere, not enough to liquefy it so that it will run. One now has a culture chamber arranged as shown in Fig. 25, with a space something less than one millimeter deep between the coverglass and the slide. A tube of agar is melted, cooled, and inoculated with spores of the mold to be studied. With a sterile capillary pipette some of the agar is transferred to the edge of the coverglass and allowed to run under until part of the space has been filled, as shown in the illustration. If the slides are prepared just before use, they will have been sufficiently sterilized by the heat used in their preparation. They may then be incubated in a glass staining jar containing moistened blotting paper in the bottom. The lid of the jar should be sealed with vaseline or surgeon's plaster. After growth has occurred, the arrangement of both the aerial and submersed mycelium may be readily seen. Photomicrographs taken from such slide cultures are shown in Fig. 26 and Fig. 49.

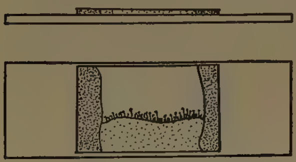

FIG. 25.—Method of growing molds between a slide and coverslip.

Isolation of Yeasts.—In general, yeasts may be isolated and cultivated on the same media as the molds. They prefer an acid medium with lots of sugar. Where "synthetic" media are used, ammonium salts must serve as the source of nitrogen since the common yeasts, at least, are unable to utilize nitrates.

The greatest difficulty with yeasts is encountered in separating them from molds, since the latter tend to spread over the surface of plate cultures and overgrow the yeasts. Sometimes one may take advantage of the fact that molds tend to float on the surface of liquid media, while yeasts tend to grow at the bottom. By inoculating material containing both in a flask arranged with an outlet at the bottom, one may, after the yeasts have grown, draw off some of the liquid at the bottom and thus obtain the yeasts

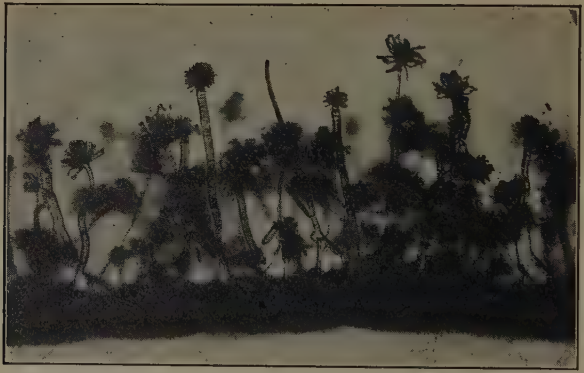

FIG. 26.—*Aspergillus glaucus,* showing appearance of conidiophores as seen in a slide culture of the type shown in Fig. 25.

relatively free from molds; this liquid may then be plated with a good chance of obtaining some pure yeast colonies.

In identifying yeasts, morphological characters are most important. The morphology is best observed in unstained wet preparations, since drying and fixing tends to shrink and distort the cells, and the size and form are the most important morphological characters. The characteristic vacuoles and volutin granules are best demonstrated by "vital staining" with a very weak solution (0.1 per cent) of neutral red. Nuclei can generally be seen only after careful fixation and staining with iron haemotoxyline. Spores may be stained by the same procedure used for bacterial spores.

Sporulation of Yeasts.—Observation of the spores is essential in identifying yeasts. Many species will not form spores readily on ordinary media. A number of methods have been devised to force yeasts to sporulate. Of these the plaster block method is most commonly used. Plaster of paris mixed with water is put in small dishes and allowed to harden. When set it is removed and placed in a larger dish or a large test-tube, and sufficient water or dilute (0.1 per cent) peptone solution added to thoroughly moisten the block but not cover its surface. This is then sterilized in the autoclave. A young culture, say not over twenty-four hours old, is used for inoculation. A generous loopful of the growth is spread over the surface of the plaster. The temperature of sporulation is critical, and with unknown species it is advisable to prepare several blocks and incubate them at different temperatures, say 20°, 25°, and 30° Centigrade. After twenty-four hours spores should be searched for by microscopic examination. Other materials, as pieces of clay flower pots, or blotting paper, may be used in place of the plaster, the idea being to maintain a limited amount of both nutrition and moisture, though there is some evidence that the calcium sulphate has some specific action in the process.

Sporulation in yeasts is said to occur quite regularly on Gorodkowa's medium, which has been widely used. The composition is:

Glucose	0.25 gm.
Sodium chloride	0.50
Meat extract	1.00
Agar	1.00
Water	100.00 cc.

Recently a new medium for spores in yeasts has been developed by McKelvey,[6] which seems to be more certain than the plaster block method. This consists of a weak carrot infusion agar to which a little calcium sulphate (plaster of paris) is added just before it is tubed. The agar is tubed, sterilized and slanted like any other agar medium. On this medium most yeasts grow fairly well, and in the author's experience form spores more readily than with other methods. Spore formation with yeasts is, however, always uncertain, and a strain should not be considered non-sporogenous until several trials have been made with different methods.

Giant Colonies.—The form of the colonies on agar is frequently of some importance with yeasts, especially of large isolated colonies which have been allowed to grow for some time. Such "giant colonies" may be obtained by just touching the inoculating wire to the middle of the surface of the agar in small Erlenmeyer flasks. It is desirable to have considerable agar in the flasks so that it will not dry in the course of several weeks, and it may be necessary to incubate the flasks in a moist chamber. If Petri plates are used, they should be incubated in cans which can be sealed with a strip of surgeon's plaster. In making cultures for giant colonies, extreme precautions must be taken to prevent contamination, especially with mold spores.

Isolation of Actinomycetes.—In studying Actinomycetes it must be borne in mind that they cannot tolerate an acid medium. The acid-sugar media recommended for yeasts and molds cannot therefore be used. They will for the most part grow on the same media as are used for bacteria, and their growth is generally stimulated by the addition of sugar.

In isolating these organisms the greatest difficulty is experienced in separating them from bacteria, since the latter for the most part grow more rapidly, and if they form spreading colonies, will overgrow the plates and suppress the Actinomycetes. But most of the Actinomycetes can grow with very small amounts of nutrient, and one may use a very weak medium on which many bacteria will not grow (and those that do will not spread much).

For general isolation of saprophytic species of Actinomycetes, perhaps Czapek's solution agar is the best. On it many bacteria will not grow and those that do are not spreaders. Some difficulty is experienced with spreading molds on this medium. Czapek's medium has the further advantage that the pigments which are quite important in identifying Actinomycetes are developed on it to a greater extent than on richer media. Soil extract agar may also be used for isolation.

Although such weak media will serve for the isolation of saprophytic soil strains, they will not permit the isolation of most pathogenic species. Many of the latter are cultivated with great difficulty; in fact, in most cases of actinomycosis of the "lumpy jaw" type they cannot be cultivated at all. These latter are fairly strict anaerobes when first cultivated, and may not grow unless serum be added to the medium.*

Culture Media for Actinomycetes.—Once isolated, most of the Actinomycetes will grow fairly well on any of the ordinary media. For their identification, cultural characters on media of rather precise composition are of great importance. Waksman[13] recommends the following as "routine" media for actinomycetes:

(1) Modified Czapek's agar (see page 28).

(2) Krainsky's glucose agar, composed of:

Glucose	10.0 gm.	Asparagin	0.5 gm.
Dibasic potassium phosphate	0.5 gm.	Agar	15.0 gm.
		Water	1000 cc.

(3) Conn's malate glycerine agar, composed of:

Calcium malate	10.0 gm.	Ammonium chloride	0.5 gm.
Dibasic potassium phosphate	0.5 gm.	Glycerine	10.0 gm.
Agar	15.0 gm.	Water	1000 cc.

(4) Citrate glycerine agar, composed as above, save that calcium citrate replaces calcium malate.

(5) Gelatine, 15% in distilled water, reaction not adjusted. Incubate 30 days at 16–18 C.

(6) Milk.

(7) Potato plug.

(8) Starch agar.

(9) Nutrient peptone agar.

Morphology of Actinomycetes.—For studying the morphology of Actinomycetes one may use the same methods that serve for the larger molds, but encounters difficulties due to their extreme minuteness. The very highest magnifications must be used to obtain a clear picture. The slide culture method may be used to good advantage. Here it is particularly important to use a medium poor in nutrients in order to prevent too dense massing of the mycelium. For observing the spores and sporophores the method of Drechsler[2] has some advantages. It consists in moistening a coverglass with a thin film of albumen. This is then lightly dropped on to the surface of a sporulating colony and gently lifted off again. The spore-bearing filaments will adhere to the coverslip and will be pulled away from the colony, but will retain their normal arrangement. They can then be fixed, stained and mounted.

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* On the other hand I recently cultivated a strain which does not grow if serum is added, though it will grow on veal infusion agar without serum.

Examination of Exudates.—In searching for yeasts, molds and Actinomycetes in pus or other exudates or tissues from cases of infection in man and animals, certain general facts must be kept in mind. Where it is possible to obtain pus from abscesses which have just been opened surgically before they have formed a fistula communicating with the exterior, it is usually relatively easy to find the organisms. After they have broken open, however, there is always an extensive secondary infection with bacteria, and it is often almost impossible to find the fungi. In such cases where the parasites cannot be obtained in the pus draining from the abscess, they may be found in sections of tissue removed from the wall of the abscess. A "biopsy" is thus frequently of great diagnostic value.

Fungi may be demonstrated in sections of tissue by the Gram-Weigert method or one of its various modifications. Unna (jun.)[11] has recently described a modification of the Unna-Peppenheim stain which is said to give a very clear picture, especially in skin sections. The sections are removed from water and stained 5–10 seconds in the following solution:

Pyronin	0.9 gm.
Methyl green	0.1 gm.
Alcohol (96 per cent)	9.0 cc.
Glycerin	10.0 gm.
Aqueous phenol ($\frac{1}{2}$ per cent) to	100.0 cc.

These sections are then rinsed in water and quickly dehydrated in absolute alcohol, cleared in xylol and mounted in balsam. Fungus elements stain red, nuclei and leucocytes appear bluish-green.

In general, fungus parasites are not so numerous in exudates as are bacteria. One must therefore not be satisfied with a hasty examination, but must patiently go over many microscopic fields. The fungi are all Gram-positive and may be found in films fixed and stained by Gram's method. But their morphology is so characteristic in the unstained state that wet preparations are to be preferred. Since the pus cells and fibrin in pus tend to obscure the field and make the finding of the fungi difficult, it is usually best to first add to the pus a little strong solution of sodium hydroxide (20 per cent) which, after some minutes, will dissolve most of the tissue elements, or at least make them more translucent, and will usually not dissolve the fungous elements.

In the case of the dermatomycoses the fungi are usually to be found in or on the hairs or scales of desquamated skin. In such cases treatment of the material with a strong alkali solution before examination is indicated. A 25 per cent solution of antiformin such as is used in digesting tuberculous sputum may be used in place of the sodium hydroxide solution.

In searching for fungi in wet preparations of pus, fat droplets are frequently mistaken for yeast cells and strands of fibrin for mycelium by beginners. Fat droplets are always more refractile than yeasts and have no granular internal structure; they may imitate budding. Mycelium also will have an internal structure not possessed by fibrin, though the mycelium of Actinomycetes may very closely resemble fibrin.

Cultivation of Pathogenic Fungi.—There is no satisfactory all-round method for cultivating pathogenic fungi. Where there is extensive secondary infection with bacteria, isolation in some cases may be impossible. Not all of the pathogenic fungi will tolerate a highly acid medium. The success in isolation is proportional to the number of cultures made. With an unknown fungus parasite one should make a large number of cultures on a variety of media. The dextrose-tartaric agar and Sabouraud's medium will prove most useful.

Extreme care should be taken to make these cultures as aseptically as possible, for mold spores are very common in the air, and mold contaminations occur much more frequently than the average bacteriologist suspects, because they do not develop in the time usually taken for observing plate cultures of bacteria. But pathogenic fungi are in general slow growers, and may require several weeks incubation before any growth is apparent.

In making cultures from the dermatomycoses, infected hairs or particles of epidermis are divided by fine sterile scissors into small particles, and these are transferred by a needle to different points on the surface of an agar plate. Many such cultures should be made in the hope of obtaining some that will be pure.

In some cases where the pathogenic fungi form aerial mycelium, they may be separated from contaminating bacteria by carefully transferring some of the aerial mycelium or spores without touching the surface of the agar.

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CHAPTER III

BIOLOGICAL ACTIVITIES OF THE MOLDS

Molds, yeasts and Actinomycetes are of economic importance because of their enzymes and toxines. They are a cause of spoilage of foodstuffs and other organic products of industry, they play an important part in the circulation of the elements of organic matter in nature (and therefore in maintaining soil fertility), they bring about chemical reactions of value to man (and are therefore important as industrial ferments) and they produce disease in man and animals. Their activity in producing plant disease lies outside the domain of this work.

As far as we know, none of the higher fungi are autotrophic, i.e., able to obtain the energy necessary for their growth by the oxidation of inorganic substances. And since they are devoid of chlorophyll, they must live upon organic matter, either as saprophytes or parasites. They cannot grow, therefore, except upon substrates which are at least in part organic, though many will grow if only an organic source of carbon is supplied as a source of energy.

Nutrition of Molds.—The molds obtain their food by simple absorption of material in solution through the cell walls of the submersed mycelium. If they are growing on a solid organic substrate, they must first secrete into this substrate enzymes capable of dissolving it. We find many forms thus secreting enzymes capable of dissolving such substances as proteins, starch or cellulose. The food so absorbed may be still further decomposed or oxidized or reduced within the cells, or may be built up into more complex substances (protoplasm, reserve food material or specific secretions as enzymes or pigments) through the activities of intracellular, or endo-enzymes.

The molds grow more slowly than do the bacteria. Consequently we do not generally find them growing in conditions where they have to compete with the latter. But where conditions are unfavorable for bacteria, there will nearly always be found one or more species of mold capable of developing. Thus we find them growing abundantly on starchy foods which are not favorable to most bacteria, and in foods containing a high percentage of sugar which will inhibit bacteria because of its high osmotic pressure, and in very acid materials, as fruit juices or sour milk.

Although they grow slowly, they may develop on materials which we would ordinarily consider as supplying very little nutrition, like tanned leather or linen or cotton cloth, if these are damp enough. In fact, molds may develop in the most surprising situations, and may grow on very unusual substrates. They not infrequently appear in laboratory reagents of various kinds, where small traces of organic matter may be present, sometimes in solutions in which one would think life would be impossible. Thus molds are quite versatile in their ability to adapt themselves to particular environments, and it is difficult to make generalized statements without noting numerous exceptions.

Moisture Requirements.—Moisture is of course requisite for growth, since molds must absorb food in solution. They can get along, however, with much smaller amounts of water than are required by bacteria, and in particular can grow in solutions of much higher osmotic pressure. Thus they may be found forming scums on the brine solutions of pickling vats, or on the surfaces of hams or other salt meats; it is well known that they will grow on syrups and jellies which will not permit a growth of bacteria.

Relative humidity of the atmosphere was found by Thom and Shaw[54] to be a critical factor for the growth of molds on butter, very little growth taking place if the humidity was below 70 per cent. On the other hand, Lewis and Yesair[40] found humidity had little effect on the growth of mold on frankfurters, probably because the substrate contained sufficient moisture.

Oxygen Requirements.—There are probably no strictly anaerobic molds, and the great majority are strictly aerobic; but a few species, notably some of the Mucors, may grow to some extent under a reduced oxygen tension.

Temperature Relations.—The different species vary markedly in their temperature requirements. In general it may be stated that the optimum temperature for most species lies somewhere near 30° C., but that some growth will take place at temperatures considerably below this, and that most forms may grow somewhat at temperatures up to 37° C. or even higher. Most species of

Penicillium have their optimum temperature between 20° C. and 25° C. and may fail to grow at temperatures above 30° C., whereas with many species of Aspergillus the optimum temperature will be around 35° C., and one species pathogenic for birds (*A. fumigatus*) finds its optimum at 40° C. The thermal death points also vary markedly with the species. Spores are of course much more resistant than vegetative mycelium, but not nearly so resistant as the spores of bacteria. Thom and Ayres[53] have studied the heat resistance of mold spores with regard to pasteurization of milk. A temperature of 145° F. (62.8° C.) for 30 minutes was sufficient to destroy practically all. Flashing for 30 seconds, a temperature of 165° –175° F. (73.9°–79.4° C.) was necessary to obtain an equal degree of sterilization. Lewis and Yesair[40] found that 140° F. for 5 minutes was sufficient to kill all of the molds from meat products which they studied. With dry heat, of course, higher temperatures and longer time intervals are required.

Enzymes of Molds.—There are so many different kinds of fungi which will grow upon so many different kinds of substrates that it is quite evident that our knowledge of their various enzymes must be very incomplete. The following is a list of some of the more important enzymes which have been obtained from fungi: Cellulase, converting cellulose to the disaccharide cellobiose; cellobiase, converting cellobiose to dextrose; cytase, acting upon hemicelluloses and the various gums (galactans, pentosans, etc.), converting them to sugars; diastase, converting starch to maltose; maltase, converting maltose to dextrose; invertase, converting sucrose to dextrose and levulose; lactase, converting milk sugar to dextrose and galactose. Many fungi produce various proteases; thus gelatine is liquefied by the gelatinase of many species, and an enzyme like trypsin which will digest coagulated egg or blood fibrin has been extracted from some. The fat-splitting enzyme, lipase, is produced by a large number of fungi. Zymase, which converts sugar to alcohol, is produced not only by yeasts, but also by several species of molds. The milk coagulating enzyme, rennin, is formed by various molds. Catalase, oxidase and reductase have been demonstrated in various fungi.

The literature on the enzymes and biochemical reactions is quite extensive. It is, however, widely scattered, somewhat contradictory, and not easily summarized. A bibliography may be found in the book by Waksman and Davison.[64]

Most molds when growing in solutions containing carbohydrate tend to produce organic acids by fermentation. In some cases the acidity may become quite pronounced. In many cases these acids are oxalic, fumaric, gluconic, or citric, rather than the lactic or acetic acid usually formed by bacterial activity. Many molds are also capable of utilizing organic acids as a source of carbon, completely oxidizing them to carbon dioxide and water. Thus *Aspergillus niger*, when grown in sugar solution, will readily produce citric and oxalic acids, make the medium quite acid in reaction, then slowly utilize the acid for further growth, causing the reaction to return toward neutrality. Molds are sometimes a cause of spoilage of foodstuffs, which depend upon their organic acid content for preservation from bacterial decomposition. They are thus found frequently in vinegar and pickling solutions, in sour milk, and on sourkraut and silage.

Like the enzymes of bacteria, the enzymes of yeasts and molds are specific for the species, and may be utilized to a certian extent for identification; but in most cases they have as yet been incompletely investigated. Sugar fermentations are, however, relied upon to a considerable extent in identifying and classifying yeasts and certain yeast-like fungi, the Moniliae.

Industrial Uses of Molds.—Some of the enzymes of molds are used commercially. The industrial applications of molds have been reviewed by Herrick and May.[30] Some of them will be discussed in some detail later in connection with particular species, but their uses will be briefly catalogued here. The diastatic action of molds has long been made use of in Oriental countries for the conversion of rice starch to sugar preparatory to alcoholic fermentation, just as we have used malt. And one species, *Mucor rouxii*, has been used to produce alcohol directly from starch, since it secretes both diastase and zymase. An extract of a mold, *Aspergillus oryzae*, containing among other enzymes considerable diastase, has been produced commercially in this country under the name "Takadiastase" as a substitute for malt and as a remedial agent in indigestion. The production of citric acid from cane sugar by a species of mold, Citromyces, has been carried on commercially; more recently certain strains of Aspergillus have been used for the same purpose with greater success. The proteolytic action of *Aspergillus oryzae* has long been made use of in the preparation of partially digested foods from soy beans in Japan, and in making the dark-brown sauce found in Chinese restaurants. The proteolytic activity of certain species of Penicillium is used in the ripening of Camembert and Roquefort cheeses. Probably many more industrial applications of the fungi will be found in the future.

Molds in Foodstuffs.—The ability of molds to grow (though slowly) at relatively low temperatures, and their ability to multiply in media of high osmotic pressure or high acidity, fits them for growth in a number of food products which have been treated in various ways to prevent bacterial decomposition. Thus we find them as important causes of spoilage in preserved fruits and jellies; pickles; butter and cheese; salted, dried and smoked meats; and stored fruits and vegetables. Storage for a sufficient length of time in a sufficiently humid atmosphere is the condition which may lead to mold development.

The absence of air and the temperatures of processing are sufficient to eliminate molds as an important problem in commercially canned foods. But, as every housewife knows, the imperfect sealing of mason jars frequently allows a mold development on home-packed fruits, jellies and vegetables. Species of Aspergillus and Penicillium are most frequently found. Of commercial products packed in sealed containers, ketchup seems to be most commonly contaminated with molds. This is apparently mainly due to the use of overripe tomatoes. If the ketchup is processed, these molds are of course dead, but observation of the mold content gives an indication of the quality of the product. Methods of enumerating mold mycelia and spores, as well as yeasts and bacteria, in ketchup have been described by Howard and Stevenson.[33] Molds may also develop in cans of sweetened condensed milk, forming small masses known as "buttons." According to Rogers, Dahlberg and Evans[51] this condition is due mainly to *Aspergillus repens*, though other molds may be responsible. They grow until the residuum of air in the product has been used up, and may be prevented by low-storage temperatures or by sealing the cans under a vacuum.

Meats.—Molds cause considerable trouble in the meat-packing industry, particularly with the various kinds of preserved meats. The growth of molds on hams, sausages and bacon is very superficial and causes no pronounced decomposition of the product, but causes marked economic loss through the expense of "reconditioning" the product and the reduced value due to its appearance. Molds and yeasts usually multiply extensively in the pickling vats. They are considered desirable by some packers, as they are supposed to take part in the chemistry of the pickling process, and to produce desirable flavors, but this is considered to be erroneous by others.

The occurrence of molds on meat products appears to be largely determined by the degree of care in handling and the humidity of the storage rooms. Lewis and Yesair[40] isolated the following species from various meats, pickling solutions, and the walls or containers of the packing plants: *Mucor racemosus*, *Rhizopus nigricans*, a species of Mortierella, *Oidium lactis*, *Monilia sitophila*, *Monascus purpureus*, *Aspergillus glaucus*, *Aspergillus niger*, *Aspergillus clavatus*, *Penicillium expansum*, *Alternaria tenuis*, and a species of Fusarium. These were studied with regard to their temperature relations, their ability to adhere to and stain sausages, and the effects of various humidities, as well as their resistance to sodium hypochlorite and ozone.

Butter.—Of the various dairy products, butter is most subject to mold spoilage, although *Oidium lactis* and *Scopulariopsis* (*Penicillium*) *brevicaulis* are frequently a cause of undesirable flavors in cheese. Moldiness in butter is largely dependent on the initial contamination of raw materials, on manufacturing procedures, and on the temperature of holding. Macy and Combs[42] found the sources of contamination (in order of descending frequency) to be the raw cream (which was always contaminated), dry parchment, piping and pumps of the creamery, water, starter cultures, and salt. Thom and Shaw[54] recognize three main types of moldiness, the smudged type with dark or smoky areas due mainly to species of Alternaria and Cladosporium; the green type, due to Penicillia; and the Oidium type, with patches of yellow or orange discoloration caused by *Oidium lactis*.

Fruits.—A consideration of mold spoilage of stored fruits and vegetables carries one into the domain of plant pathology, for some of the rots of such products are due to organisms which have invaded the fruit on the living plant, though even with those which invade during storage we must remember that the plant tissues are alive, and presumably possess a defense mechanism which can be overcome only by certain species having to a certain degree parasitic qualities. It is not surprising therefore that we find some species of fungi occurring characteristically on each kind of fruit or vegetable. Thus various fruits, especially the plums and cherries, are spoiled by pathogenic species of Sclerotinia, which also cause disease in the trees; the common brown soft spots in stored apples are most frequently produced by *Penicillium expansum*, and the spoilage of citrus fruits is mainly due to *Penicillium digitaum* and *Penicillium italicum*. The spoilage or "leak" of strawberries, as well as the soft rots of both white and sweet potatoes, are caused by *Rhizopus nigricans*.

Molds in Soil.—The mold flora of the soil, and the function of fungi in soil transformations, have been extensively studied by Jensen,[37] Gilman and Abbott,[24] Janke and Holzer,[36] and especially by Waksman and his co-workers,[57-63] whose papers must be consulted for detailed information.

Some 265 species of molds have been isolated from soil. Those most commonly found, however, are species of Zygorrhynchus, Penicillium, Trichoderma, Fusarium, Mucor, Aspergillus, and Rhizopus, the frequency being in the order given. Quantitative estimations of soil fungi are difficult because by the ordinary plating method one enumerates spores as well as mycelial fragments. And as spores are formed in enormous numbers by many fungi and may remain quiescent for long periods of time, plate counts may give quite erroneous impressions of the number of fungi active in a given sample of soil. In general, however, the number of molds that develop on plates must bear some relation to the number active in the soil.

The number of fungi in soil increases with the content of the soil in organic matter. Because they can tolerate high degrees of acidity, molds are much more numerous than bacteria or Actinomycetes in acid soils; they are by no means lacking, however, in soils that are neutral in reaction. A soil receiving ammonium sulphate as fertilizer with a reaction of pH 4.2 contained 129,000 fungi per gram. The same soil limed (pH 5.2) had only 32,000. Fungi occur at all depths in soil, at least up to 4 feet, though the numbers drop rapidly after the first 6 inches.

Soil fungi play two important roles in maintaining soil fertility. They readily decompose complex organic substances, especially cellulose, starch and proteins, thus rendering the elements contained in these compounds available to plants as food; and they assimilate soluble inorganic nitrogen compounds and minerals, thus removing them temporarily from soil solution, and so prevent their leaching out when present in excess over the needs of green plants.

The investigations of Waksman and Skinner[65] have shown that under aerobic conditions the decomposition of cellulose in the soil is almost entirely the work of molds; only under anaerobic conditions which seldom obtain in a well-cultivated soil do bacteria play an important part. The amount of nitrogen available to the fungi is frequently the limiting factor of cellulose decomposition. The soil fungi are also very active in ammonification. Many of the molds will produce ammonia from proteins more rapidly than the most active of the ammonifying bacteria. Thus the molds are among the primary agents concerned in the decomposition of dead plant matter in the soil. On the other hand, a large part of the material decomposed is built up into mold mycelium. A considerable proportion of the organic matter of the soil consists of this mycelium. It may be again rendered available to green plants upon the death and disintegration of the fungi.[29] Thus the molds tend to stabilize the supply of plant food in the soil.

Mycorrhiza.—In connection with soil fungi, mention should also be made of a relationship between certain fungi and the roots of higher plants, called the mycorrhizal relationship. It is found that the roots of many plants are invested by a fine network of mycelium (ectotrophic mycorrhiza), which in some cases also penetrates the root tissues (endotrophic mycorrhiza). Many different kinds of plants may have such a growth of fungus mycelium on or in their roots, and a number of different kinds of fungi have been found in such an association with higher plants. The relationship is in most cases a symbiotic one, the association being of benefit to both the plant and the fungus; in some cases the investing mantle of mycelium serves in place of root hairs to absorb moisture and minerals in solution which are given to the host plant in return for carbohydrate supplied the fungus. The fungus mycelium also serves to decompose organic matter in the neighborhood of the plant roots. In some cases this symbiotic relationship is apparently obligatory; it is claimed that orchids are incapable of growing without their mycorrhizal fungi. For an extensive review of the mycorrhizal fungi with bibliography, see Waksman.[56]

Fungus Diseases in Man and Animals.—A great variety of different molds have been described as causing various diseases in man and animals. Some of these are definitely known to bear such an etiological relationship, but in surveying the literature on pathogenic fungi one cannot fail to be impressed by the degree to which Koch's postulates have been ignored. Many of the fungus diseases are quite superficial lesions of the skin or mucous membranes. Mold spores are so ubiquitous that it is not at all surprising that they may frequently be cultivated from skin lesions or wounds, from pus or sputum. Yet very often the mere presence of a fungus in an exudate or culture has been considered sufficient evidence to warrant describing it as a cause of disease. One must therefore develop a degree of healthy skepticism with regard to the pathogenic fungi.

In addition to monographs dealing with particular groups or species, rather extensive works on pathogenic fungi have been published by Plaut and Grütz,[48] Sartory,[52] Brumpt,[10] and Castellani.[12]

Although fungus infections in man and animals are not nearly so common as bacterial diseases, they are none the less important and interesting. Fungus infections are referred to as "mycoses," frequently with a prefix indicating the part affected, as "otomycosis" (ear), "pneumonomycosis" (lung), etc., and often followed by an adjective indicating the causative organism, as "Otomycosis aspergillinae" (an infection of the ear with Aspergillus).

Before making any generalized statements about fungus infections, it should be noted that there is a highly specialized group of parasitic fungi producing certain skin diseases or "dermatomycoses" (favus, the ringworms, etc.), to which such statements will not in general apply. Unlike most of the other fungus parasites of animals, these (so far as we know) have their natural habitat on the infected man or animal and do not occur free in nature. The infection is contagious, being transmitted by direct contact from one individual to another. It almost invariably remains localized in the skin, not tending to spread through the body, and does not endanger life. The fungi producing these dermatomycoses belong to a large number of different species which can only be differentiated and classified with some difficulty. They will be briefly discussed in a later chapter.

In a general way the fungus infections are milder and more chronic than bacterial diseases. But they tend to persist for a long time, to spread progressively, and eventually to endanger life by metastatic lesions in internal organs.

The fungus diseases are for the most part not contagious. In many cases it is hardly possible to produce the infections experimentally in laboratory animals. One may, for instance, sometimes inoculate healthy cattle directly with pus containing the parasite of bovine Actinomycosis from infected cattle without any infection occurring. In some cases we have clear evidence that the infecting organisms are derived either from soil, or (more frequently) from vegetable matter upon which they live as saprophytes, and this will probably be found eventually to be true of most of the others. This is supported by the fact that a large proportion of human cases occur in farmers, stablemen and others engaged in occupations where they are exposed to dust and particles of dried vegetable matter; by the finding of pathogenic species of fungi growing saprophytically upon vegetable matter, as straw and grain; and by direct demonstration of particles of such material in the tissues in primary lesions.

In a few cases the fungus diseases exhibit a limited geographical distribution. Thus the disease known as "blastomycosis" is practically unknown outside of North America, and the great majority of cases have occurred in the upper Mississippi and Missouri valleys; sporotrichosis is also more prevalent in these areas than in other parts of the United States. Coccidioidal granuloma is rarely found except in the San Joaquin valley of California. This limited distribution has not been satisfactorily explained. It has been suggested that the parasites causing these diseases are also parasitic on certain plants found only in those localities.

Some species of pathogenic fungi, as *Aspergillus fumigatus*, are very common and widely distributed, yet infections by them are very rare. It would appear then that these molds are not, essentially, human or animal parasites, and that infection occurs only in occasional individuals of unusually low resistance (as thrush in undernourished infants or adults debilitated by some wasting disease) or in individuals whose occupations expose them to extraordinarily heavy infection (as aspergillosis in the "gaveurs des pigeons," page 104).

As with bacterial diseases, the pathogenic fungi have various portals of entry to the body which are to a certain extent specific.

Thus we have the group mentioned above which grow on the skin or hairs; others, like the thrush organism, are primarily parasites of mucous membranes, causing diseases of the mouth or vagina. Some cases are primary in the lungs, the organisms being inhaled. There is a group of fungi which develop characteristically in the external ear, while with some species it is probable that the organism usually can develop only when introduced directly into the tissues through a wound.

Most species have a very low invasive power. In fact, in some of the so-called fungus infections we do not have an infection in the strict sense at all, for the parasites do not invade the tissues but merely grow on the body surface. This is true of some of the dermatomycoses; and in many cases of so-called otomycosis the fungus does not invade the epithelium of the ear, but merely grows as a saprophyte upon the ear wax. In the dermatomycoses and most cases of thrush the fungi merely penetrate the upper layers of epithelium.

Pathologic Anatomy of Fungus Infections.—In some of the fungus diseases, however, extensive and deep-seated lesions may occur, and the disease may spread to other parts of the body by metastasis either by way of the lymph vessels or the bloodstream. The tissue changes brought about in these cases are in part of the nature of abscesses, and in part of the nature of granulomata. Immediately surrounding the parasite there is usually death of the tissues, with softening, an accumulation of leucocytes, and the formation of pus. But these abscesses become surrounded by a dense layer of new fibrous tissue infiltrated with mononuclear luecocytes and sometimes containing giant cells. The degree to which one or another of these processes predominates varies with the virulence of the strain and the mode of infection. Thus the primary lesions of "blastomycosis" in the skin are mainly granulomatous, the secondary lesions following a bloodstream distribution are usually pure abscesses. In animals experimentally inoculated with rather large doses, pure abscesses similar to those produced by the pyogenic cocci usually develop. But in some human infections the lesions may so closely resemble tuberculosis or syphilis, or even cancer, as to make the diagnosis difficult. In experimental infections in laboratory animals the type of lesion may vary widely with the dosage and mode of inoculation. Thus with *Aspergillus fumigatus* spores injected intravenously into pigeons, if the doses are large there will develop areas of necrosis with haemorrhage and very little cellular infiltration about the germinating spores, but with very small doses one gets typical tubercles in internal viscera (see Fig. 27) after a much longer time; or, if one blows a mass of spores into the trachea, there develops an acute pneumonia fatal within twenty-four hours, whereas if the pigeons are fed on infected grain so that only an occasional spore is inhaled from time to time, there are found cavities in the lungs and a massive growth of mycelium in the air sacs.

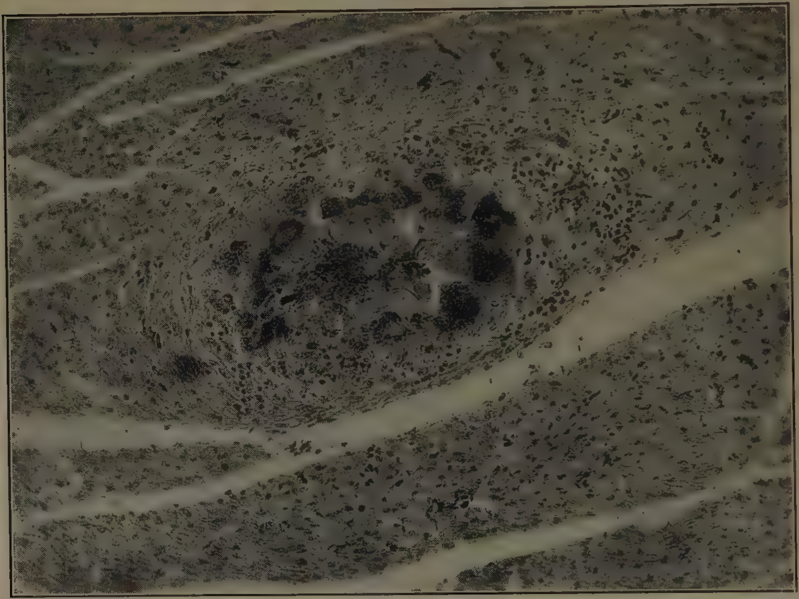

Fig. 27.—Histological tubercle in the heart muscle of a pigeon inoculated intravenously with spores of *Aspergillus fumigatus*.

Toxins of Fungi.—It is not clearly understood how the pathogenic fungi injure the tissues. Failure to demonstrate toxins, either extra- or intracellular, has led to a general acceptance of the idea that the injury is primarily mechanical, the fungus mass merely acting as a foreign body. But, while the fibrosis and giant cell reactions about some lesions bear a resemblance to a foreign body reaction, the extensive necrosis and suppuration which occur in the center of most lesions cannot be readily explained in this way. Moreover, the experimental lesions produced with freshly isolated and highly virulent strains of some species, as *Aspergillus fumigatus* and *Monilia albicans* are so acute that there can be but little doubt that the disease is produced by the same mechanism as that found in bacterial infections. A pigeon inoculated intravenously with a suspension of spores of *A. fumigatus* may die within twenty-four hours, showing at autopsy multiple punctate haemorrhages, and areas of degeneration or necrosis in parenchymatous tissues, in short, just such a picture as may be produced by a highly virulent streptococcus. Such findings naturally suggest that a potent toxin is formed.

Considerable investigation has been carried on with regard to the toxins of the pathogenic fungi, but it is largely contradictory and inconclusive. No exotoxins have been definitely established. The occurrence of endotoxins is of considerable theoretical interest. Our conception of endotoxins is largely based upon the demonstration of endo-enzymes by Buchner's classical experiment. But it is not possible to express the "cell sap" of bacteria by pressure, as Buchner did with yeasts, because of their minute size. All other attempts to remove toxins from within the cells of bacteria, by chemical extraction, autolysis, grinding (after drying or freezing) may be criticized as introducing factors that might readily alter the toxins. But the cell sap of molds may be readily expressed in a Buchner press. It is remarkable that very few attempts to obtain a toxin from pathogenic fungi by this method are recorded in the literature.

Gortner and Blakeslee,[25] during the course of some experiments dealing with antibody production, accidentally discovered that the cell sap of *Rhizopus nigricans* is highly toxic to rabbits. Since this observation is the only available demonstration of an endotoxin, it may be considered in some detail. The toxic substance was obtained both by expressing the juice of the mold[3] and by extraction of dried mycelium with hot water. It gave the reactions of proteins, was non-dialyzable and precipitated by alcohol. Intravenous injections in rabbits produced almost immediate death, with both symptoms and autopsy findings "practically indistinguishable from anaphylactic intoxication." Subcutaneous injections gave rise to abscesses which broke down to form ulcers. The material was quite harmless by mouth.

The author has, however, failed to confirm these experiments. Three different strains of *Rhizopus nigricans* have been used, one an old laboratory strain, the others freshly isolated from a strawberry "leak" and from a rotten potato respectively. They were grown on 5 per cent dextrose, 1 per cent peptone solution until an abundant mycelium had developed. This was then separated from the liquid by straining through gauze, wrung as dry as possible by hand, triturated with fine sand, and expressed in a Buchner press at 100 kg. per sq. cm. The juice was filtered through paper and injected intravenously into rabbits (three with each strain in doses from 1 to 5 cc). No immediate effects were noted save temporary dyspnoea, and all rabbits survived. Several of these were given a second injection after two weeks and developed typical anaphylactic reactions, one dying.

It is quite possible that the results of Gortner and Blakeslee were due to their using a lot of rabbits which had somehow or other become hypersensitive to the proteins of this mold.

A number of investigators have been concerned with the toxins of *Aspergillus fumigatus* because of the high and constant virulence of this organism for laboratory animals. Renon[49] was unable to find any toxic substance either in the medium or by extracting the mycelium with various solvents. Similar results were obtained by Kotliar,[39] Macé,[41] and by Martins,[43] although the latter author did observe death with one rabbit inoculated with spores heated to 100° C.

On the other hand, Ceni and Besta[13] found in extracts of *Aspergillus fumigatus* a heat-stable toxic substance affecting mainly the nervous system, and this was confirmed by Bodin and Gautier.[8] Bodin and Lenormand[9] studied the matter further and concluded that there were two toxins, one a substance soluble in fat solvents which produced convulsions and a rapid death in rabbits, the other a volatile substance obtained by distillation which produces paralysis and death in guinea pigs. Novak, working in the author's laboratory, has failed to find either of these substances. In any case these toxins, if they exist, probably have nothing to do with aspergillosis, since they produce only nervous symptoms which are lacking in that disease, and since they have no effect upon pigeons, which are the animals most susceptible to experimental infection.

A number of attempts have been made by Novak and the author (unpublished work, still in progress) to obtain toxins by expressing the cell sap of *Aspergillus fumigatus*. Two strains isolated from spontaneous infections in ruffed grouse have been used; both proved to be highly virulent by animal inoculations. They have been grown on dextrose-peptone solutions, using several different brands of peptone. In some cases the medium has had calcium carbonate added to maintain a neutral reaction; in other cases alkali has been added before expressing. In some cases the mycelium has been frozen and thawed before expressing. We have had for the most part negative results. A few animals, both rabbits and pigeons, have died or have become markedly ill, but always within a short time after inoculation, and, with the rabbits at least, the symptoms have been those of an anaphylactic shock. We assume that occasional animals are naturally hypersensitive to some protein present in the cell sap.

One may find other scattered references in the literature to possible toxins extracted from pathogenic fungi, none of which are very convincing.

Toxins of Mushrooms.—A word or two may be properly interpolated here concerning the toxins of the poisonous mushrooms. These vary with different species. A survey of the mushroom poisons has been presented by Ford.[22] In the case of the fly amanita (*Amanita muscaria*) the poison is an alkaloid, muscarin. Considerable work has been done on the poisons of the most deadly of the mushrooms, *Amanita phalloides* (Fig. 28). The work of Ford[19] has established that there are two poisonous substances present. One is a haemolytic agent called "phallin," the other a general toxin known as "amanito-toxin."[20] The former is easily inactivated by heat, the latter not. Laboratory animals may be immunized with extracts of the fungi, their serums developing both anti-haemolytic and antitoxic properties.[19] The haemolytic substance has been isolated and is said to be a pentose-containing glucoside.[1] It is, however, contrary to the opinion of most immunologists that a non-protein substance may give rise to antibodies. The "amanito-toxin" alone did not give rise to protective antibodies in Ford's experiments.[21] It was found to be neither a proteid, alkaloid, glucoside, nor a conjugate sulphate.[21, 23]

Contrary to the experience of Ford, de la Riviere[16] found that the toxin affects mainly the nervous system, spasmodic convulsions being the most prominent symptoms. Both authors agree, however, that the toxin is not bound by nerve tissue. A wide variety of animals, from monkeys to fish, are susceptible. Sheep are apparently immune to poisoning by mouth, but succumb to injections. De la Riviere succeeded in obtaining an antitoxin (with the whole extract) by immunizing horses. This not only protected laboratory animals but was shown to have some therapeutic value in cases of human poisoning.

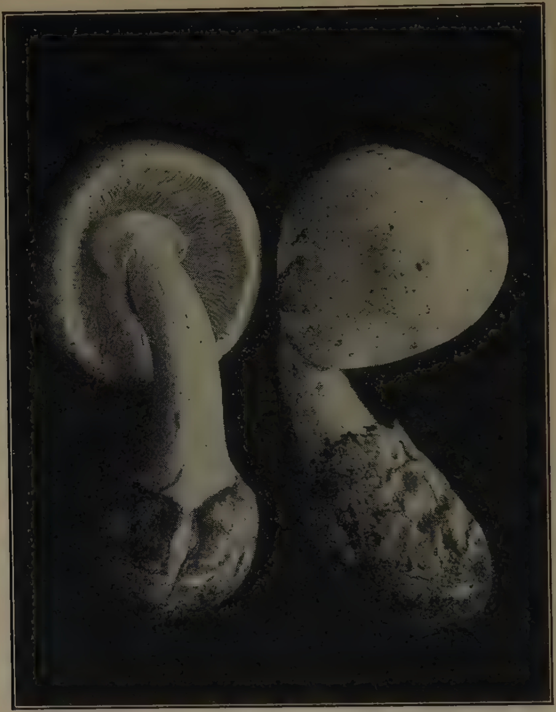

Fig. 28.—*Amanita phalloides*, the "Death Cup." The cup-shaped envelope (volva) about the base of the stem, and the ring or collar about the stem near the cap are distinguishing signs. (White varieties, of which the above are an example, are sometimes known as *A. verna.*)

Green and Stoesser[26, 27] have studied the effect of sodium ricinoleate upon the toxins of *Amanita phalloides*. Whereas this soap neutralizes the toxins of diphtheria bacilli and scarlet fever streptococci, it augments the toxicity of the amanita poison.

Immunity Reactions of the Fungi.—The immunity reactions of the fungi have not been extensively investigated, and such work as has been published records in the main negative results. It would appear that in general fungi give rise to antibodies only in small amounts, and that the antibodies so formed are not highly specific, reacting in some cases with widely diverse types. The difficulty is probably in part, at least, due to the rather dense cell wall formed by most fungi (which is not present in bacteria) that allows only a slow diffusion of the intracellular proteins into the tissues. That this may be the case is indicated by the results of Balls[2] who obtained precipitating serums of relatively high titre and specificity with yeasts by inoculating, not the cells, but proteins liberated from them by autolysis.

Agglutinins.—Most attempts to carry out agglutination reactions with fungi have been unsuccessful. This is due in part to the difficulty in obtaining stable suspensions. Generally spores have been used, though some workers have used suspensions of ground-up mycelium.

Cummins and Sanders[14] could demonstrate no agglutinins for *Coccidioides immitis* in experimentally infected animals. Similarly Nicaud[47] could find no agglutinins for spores of *Aspergillus fumigatus* in the sera of human cases of aspergillosis, and Matsumoto[44] cannot obtain any agglutination of spores of *Aspergillus amstelodami* in the sera of intensively immunized rabbits.

On the other hand, Widal[66] and others found that the sera of sporotrichosis cases would agglutinate the spores of *Sporotrichum beurmanni* in rather high dilutions, 1–300 to 1–400 on the average, and this observation has been confirmed by numerous later authors. However, the same Sporotrichum spores are also agglutinated in rather high dilutions by the sera of thrush cases and cases of actinomycosis, according to Widal and his coworkers.[66] This observation was, however, not confirmed by Fineman[18] in the cases of thrush studied, with American strains of Sporotrichum. Widal and his coworkers found that the sera of thrush cases would agglutinate Sporotrichum spores at a higher titre than they would suspensions of the thrush parasite. Fineman could obtain agglutinins only in a very low titre for *Monilia albicans* in immunized rabbits. According to Epstein[17] this organism agglutinates spontaneously too readily to be used for agglutination reactions. Blakeslee and Gortner[4] obtained rather strong agglutinating sera for the spores of Mucors, with some cross reactions. It should be pointed out that the spores of Sporotrichum and of the Mucors have much thinner walls than do most mold spores, and also form uniform suspensions fairly readily.

Precipitins.—Very little study of precipitins has been carried on. Cummins and Sanders [14] record negative results with sera of patients and inoculated guinea pigs in coccidioidal granuloma. Michel [45] obtained no reactions with *Monilia psilosis* in cases of sprue. Matsumoto [44] obtained precipitates when the sera of rabbits immunized with species of Aspergillus were tested against broth filtrates, but not with extracts of mycelium; the reactions were not definitely specific. I have been unable to demonstrate precipitins with sera of rabbits intensively immunized with cell sap of *Aspergillus fumigatus* and of *Cephalosporium acremonium.*

Complement Fixation.—Widal and his coworkers [66] found complement fixation positive in sporotrichosis, as was also the case with agglutination, and found the same cross reactions with thrush and actinomycosis; complement fixation indeed seems to be less specific than agglutination in these cases. Epstein [17] found the complement fixation reaction positive only once in twenty cases of thrush. Cummins and Sanders [14] obtained no complement fixation in coccidioidal granuloma. Matsumoto [44] obtained more marked results with complement fixation than with precipitation in his studies of the Aspergilli, but found no correlation between the grouping of species by complement fixation and their morphological characters.

Allergic Reactions.—More significant results seem to have been obtained from a study of allergic reactions than from other phases of immunity with the fungi. In 1908 Bloch [5] established that, after an experimental skin infection with *Achorion quinckeanum* had been established in guinea pigs, the animals are immune to reinoculation over the entire skin surface. This immunity is in general not species-specific, the animal remaining also refractory to other rather distantly related species of dermatophytes. If one does succeed in establishing a second infection, the inflammatory reaction is more acute and the lesion heals more quickly than in a control animal. A similar immunity was demonstrated in human cases where deep-seated lesions occurred.

Inoculations of filtrates of old broth cultures of the fungus gave rise to a characteristic papule in human cases if deep-seated lesions were present, but not when the lesions were superficial. This allergic reaction also proved non-specific, infections with different species of dermatophytes giving reactions with the same antigen.

Further observations were reported later by Bloch and Massini,[6] among them the following interesting experiment. Skin from a patient allergic to ringworm fungi was transplanted to a non-sensitive individual. After the graft was established, it was found that this area of skin was still hypersensitive, although the "native" skin of the recipient did not become sensitized. It was also claimed that deep-seated infections healed more rapidly after establishing an artificial infection with *Achorion quinckeanum*, an observation which is confirmed by Plaut.

A number of other dermatologists have investigated the cutaneous reactions with extracts of Trichophyton fungi (called "trichophytin"). It seems to have been established that these extracts regularly give reactions in patients in whom deep-seated lesions occur, less regularly in others; that the reaction may persist for some time after the lesions have healed: and that certain normal individuals, even those in whom a previous history of ringworm cannot be established, may react; so that, as with tuberculin, a negative reaction is of more diagnostic value than a positive one. The trichophytin seems to have some therapeutic value in certain deep-seated infections, but the effect is not a specific one; there occurs a "focal" reaction in the lesion as well as the local one at the site of inoculation, and such therapeutic effect as is noted is to be attributed to this increased inflammatory reaction, being no greater than that obtained when irritants are applied locally.

The trichophytins have been prepared in various ways. According to Bloch, the best procedure is to evaporate the broth filtrate to one-twelfth its original volume, to which is added the cell-sap expressed from the mycelium. The active substance has been chemically investigated by Bloch, Labouchere and Schaaf,[7] who claim that it is a starch-like, nitrogen containing, levorotatory polysaccharide.

Trichophytide.—In connection with the allergic reactions of the ringworms, mention should be made of the condition known as *trichophytide*, first described by Jadassohn. This is a generalized skin eruption occurring during the course of a ringworm infection, similar to the generalized eruptions occurring in scrofula or other forms of tuberculosis, and designated tuberculide by Darier. It is as yet uncertain whether this affection is due to a toxic reaction of some sort (perhaps caused by an allergic state of the patient) or to a dissemination of the fungus by the blood stream. The former hypothesis would seem more reasonable, but positive blood cultures have been reported.

Cutaneous Reactions in Other Mycoses.—Cummins and Sanders [14] found cutaneous reactions inconclusive in rabbits experimentally inoculated with *Coccidioides immitis*. Jacobson [31] obtained marked constitutional and local reactions in six cases of coccidioidal granuloma, a milder reaction in a case of blastomycosis, and no reaction in four normal controls, when filtrates of broth cultures of *Coccidioides immitis* were injected subcutaneously. Experimentally inoculated guinea pigs also reacted when the infection had persisted long enough. Positive cutaneous reactions in sporotrichosis have been recorded by Bloch and others, but according to Gougerot this reaction is no more specific than the other immunity reactions in sporotrichosis. Weiss and Landron [67] tested the skin sensitivity of sprue patients and normals to filtrates of cultures of *Monilia albicans* and *Monilia psilosis*. A much larger proportion of sprue cases reacted, but about one-fourth of the normals gave reactions to both organisms. Nicaud [47] tried various extracts of *Aspergillus fumigatus* on several patients. Only a preparation made by long-continued grinding was active. It gave negative results with a normal subject and a case of localized tuberculosis; a moderate reaction with a case of advanced tuberculosis (in whose sputum later the Aspergillus was demonstrated) and a marked local and focal reaction in a case of pulmonary aspergillosis.

Asthma.—Recently considerable attention has been directed toward mold spores as possible etiologic agents in asthma. It now seems well established that asthma is due to an allergic state toward substances which may be present in the air as dust, and so inhaled. That mold spores may be the exciting agents was suggested by van Leeuven.[55] Cadham [11] demonstrated that the spores of the wheat rust organism, *Puccinia graminis*, excited asthmatic attacks in certain cases which he studied. Hansen [28] has observed a number of cases apparently due to mold spores. These cases are first detected by cutaneous reactions, but Hansen applies rather strict criteria for establishing the diagnosis, involving demonstration of the mold in the habitual surroundings of the patient, complete relief after removal from these surroundings, an immediate relapse after experimental exposure to the mold in question, and finally a positive Prausnitz-Kustner reaction (passive transfer of the skin sensitivity to a normal person). He finds various species of Aspergillus most frequently give reactions. Hopkins, Benham and Kesten [31] have reported a case due to a species of Alternaria, and suggest that an eczema from which the same patient suffered might be due to a similar cause.[32]

Treatment of Fungus Infections.—The treatment of the fungus infections is frequently unsatisfactory, the infections persisting for long periods in spite of anything that may be done. In actinomycosis, "blastomycosis," and coccidioidal granuloma the prognosis is grave and the mortality is very high indeed. With superficial infections, as the dermatomycoses and thrush, local applications of strong antiseptics may sometimes lead to a rapid cure. Many of these, however, are also stubborn and persistent. Roentgen-ray treatment of certain of the dermatomycoses is also very successful.

In sporotrichosis the internal administration of iodides causes the lesions to rapidly disappear. Similar though less marked beneficial results are claimed for the use of iodides in actinomycosis and "blastomycosis," so that it has come to be generally considered that the iodides are a specific for fungus infections comparable with the arsenicals in protozoan infections. But Davis [15] has shown for Sporotrichum (as have Reynolds and Henrici [50] for an Actinomycete), that the iodides have no effect upon the fungi themselves either in vivo or in vitro, growth occurring in media containing as much as 10 per cent of potassium iodide. Such therapeutic results as are obtained must therefore be due to an action on the tissues rather than on the parasite. The iodides probably stimulate the formation of fibrous tissue which tends to wall off the organisms.

Many attempts have been made to find other specific drugs for the treatment of fungus infections, but without much success. Probably because they have been extensively used as fungicides in the treatment of plant diseases, copper salts (and recently colloidal copper [35]) have been used. Tartar emetic and preparations of arsenic and mercury are also used. Although apparent cures and improvements under such treatments have been reported, the results in general are not sufficiently striking to indicate that any specific chemotherapeutic agent has been discovered. Recently it has been claimed that local applications of various essential oils, and particularly of thymol, are very efficacious, but their use is of course limited to superficial lesions.[46]

Many of the fungus infections, even of the more severe types, tend to remain localized, spreading by extension only, for long periods. Where medication proves useless in such cases probably complete surgical excision or even amputation is the best treatment.

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CHAPTER IV

MOLDS BELONGING TO THE PHYCOMYCETES

There are probably no Archimycetes or Oömycetes likely to be of interest to the bacteriologist.

Orders of Zygomycetes.—The Zygomycetes are subdivided into two orders, the differentiation being based largely upon the structure of the non-sexual spores, the Entomophthorales multiplying by conidia, and the Mucorales reproducing mostly by sporangiospores. The so-called conidia of these Phycomycetous fungi are probably not true conidia as are found in the Ascomycetes, as in some cases at least, careful microscopic examination shows that they are really minute sporangia containing but a single sporangiospore. Further, they are multinucleate. They are sometimes referred to as sporangiola. It is also rather difficult to sharply divide the two orders on the above-described basis, as some of the Mucorales are found to form definite sporangia with numerous sporangiospores at the ends of the sporangiophores, and conidia (or sporangiola) on lateral branches. Other species which resemble the Mucorales in all other respects (and are therefore retained in that order) reproduce only by conidia.

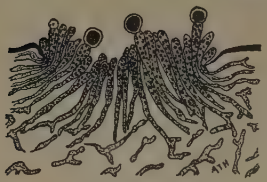

Fig. 29.—Section (stained) through the body wall of a house fly infected with *Empusa muscae,* showing the development of conidiophores and conidia. Note the multinucleate character of the mycelium.

Entomophthorales.—Members of this order will not be encountered in bacteriological work. As their name implies, they are mostly parasitic on insects. One of the best known, *Empusa muscae,* causes a disease of the common house fly. The mycelium penetrates the body tissues and forms sporophores on the surface (Fig. 29). The flies are killed by the fungus, and are frequently found in the fall on walls or windows covered with a whitish powder-like coating. When mature, the conidia are forcibly projected for a distance of several millimeters, and form a white deposit about the dead fly, especially discernible upon window panes (Fig. 30).

Families of the Mucorales.—The Mucorales are subdivided into two groups, those forming only sporangia, and those forming conidia. The former comprises (according to Lendner) four families: The Mucoraceae, with sporangia all of one kind, containing a columella, the sporangial membrane being thin and rupturing or dissolving easily; the Thamnidiaceae, with sporangia of two sorts, large ones (terminal on the sporangiophore) which possess a columella and diffluent membrane and form numerous spores, and small ones (laterally distributed) which form no columella, possess a firm membrane, and form but few spores; the Pilobolaceae, with sporangia possessing a tough membrane that, when mature, dissolves at the base and allows the whole sporangium to be forcibly discharged from the columella; and the Mortierellaceae, with sporangia like those of the Mucoraceae, save that no columella is formed. The second group contains two families: The Chaetocladiaceae, with conidia occurring singly, formed upon conidiophores which are expanded at their tips; and the Cephalidaceae, whose conidia are two-celled or multicellular, or are arranged in chains. The Chaetocladiaceae are mostly parasitic upon other

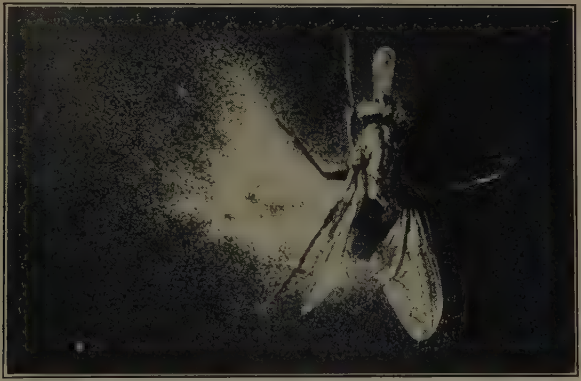

Fig. 30.—A fly dead of *Empusa muscae* infection. The white powder surrounding the fly is composed of conidia which have been discharged for some distance.

families of Mucorales, as are also some of the Cephalidaceae, though the latter are also encountered in soil (see Fig. 31).

Mucoraceae.—The great majority of the Phycomycetes likely to be encountered in bacteriological work will fall in the family of Mucoraceae. The Mucoraceae are a group of molds, frequently referred to as the bread molds, found abundantly in soil, manure, on fruits, and especially on starchy foodstuffs. They all have the same general structure and appearance and are easily differentiated from other groups of molds by the coarse, non-septate mycelium, by the abundant and loosely meshed aerial mycelium, and by the lack of distinctive colors, the spores being generally black or brown, the mycelium white or gray. In older literature the term "mold" is frequently restricted to fungi of this type, molds of the type of the Fungi Imperfecti being referred to as "mildews."

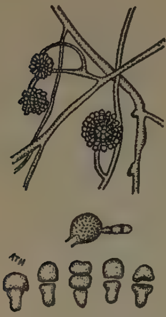

Fig. 31.—*Syncephalastrum* species, isolated from soil. Chains of two or three spores surrounded by a membrane are formed over the inflated end of the sporophore. These spores are sometimes referred to as conidia, though they are really formed in small sporangia

The Mucoraceae are distinguished from the other families of the order by the presence of a columella in the sporangium. The columella may be looked upon as a septum separating the sporangium from the sporangiophore, which has become bulged into the sporangium. In addition to sporangiospores some species also reproduce by chlamydospores which appear as round black swellings, like beads, strung on the mycelium. These may be mistaken for zygospores. Several species also characteristically break up into a series of spherical oidia when immersed in liquid where aeration is not abundant. These oidia, yeast-like in appearance, may reproduce for a while by budding, forming new round cells of the same type.

Zygospores may be formed by the fusion of neighboring filaments from the same plant in some cases (as in Zygorrhynchus) or only by fusion of filaments from two neighboring plants in others (as most of the Mucors and Rhizopus). The first type are said to be homothallous, the second heterothallous. As was pointed out in Chapter I, in the latter case there is a physiologically distinguishable sex, although the elements are morphologically identical. This very interesting fact was discovered by Blakeslee.[2, 3] Since the two sexes cannot be distinguished, they are designated as plus or minus strains rather than as male and female. Sometimes bodies resembling zygospores may be formed without fusion of the hypha with another. Such spores are called azygospores. They may be formed by an isolated plant, or only by hyphae which approach filaments from another plant of the opposite sign. It is noteworthy that plus and minus strains of different species may exhibit this evidence of sex when grown in contact, even though actual conjugation does not occur. In this connection it is also interesting to note that certain of the Chaetocladiaceae, mentioned above as being parasitic on some of the Mucorales, also exhibit plus and minus strains; and that plus strains of the parasite will only infect minus strains of the host, and vice versa. It would thus appear that their parasitism had arisen from an abortive attempt at hybridization.

The zygospores are usually large cells, generally black, and with a rough, warty exterior. The filaments which form them become expanded near the spore, forming broad supporting bands called suspensors. These are sometimes of characteristic form. Unfortunately, zygospores are seldom formed in cultures on artificial media in the laboratory. They are found most frequently on strains freshly isolated from their natural habitat. Naturally, with heterothallous varieties no zygospores will be seen unless both plus and minus strains are included in the culture. According to Blakeslee, prune extract and moistened slices of bread are media most favorable to the production of zygospores.

Genera of Mucoraceae.—The following key covers those genera of the Mucoraceae which contain species likely to be encountered by the bacteriologist.

A. The fungus spreads over its substrate by stolons or runners.
    1. Sporangiophores arise at the nodes of the stolons. *RHIZOPUS.*
    2. Sporangiophores arise at the internodes. *ABSIDIA.*

B. No stolons or runners are formed.
    1. Sporangiophores are simple or branched; sporangia borne apically on the sporangiophore and its branches.
        a. Zygospores formed from equal gametes, usually heterothallous. *MUCOR.*

b. Zygospores formed from unequal gametes, homothallous.
ZYGORRHYNCHUS.

2. Sporangia borne only on the lateral circinate branches of the sporangiophore.
    a. Sporangia globular, columella not constricted.
    CIRCINELLA.
    b. Sporangia pear-shaped, columella constricted.
    PIRELLA.

Of the various genera of the Mucoraceae, Mucor and Rhizopus contain practically all of the species commonly encountered in bacteriological work. Brief mention may be made of some of the others, however.

Absidia.—The genus Absidia is characterized by the formation of sporangiophores arising from the arched stolons themselves (rather than at the holdfasts, as is the case with Rhizopus). Further differential characters are the rounded or pear-shaped columella, which is quite different from the flattened hemispherical columella of Rhizopus, and the formation of peculiar curled filaments which surround the zygospores, arising from the suspensors. Lendner[7] recognizes seventeen species of Absidia, some of which are frequently found in soil.

Zygorrhynchus.—In Zygorrhynchus the zygospores are formed by the fusion of neighboring branches of the same hypha. This mold is therefore homothallous. But there is some apparent differentiation of the conjugating branches, one being larger than the other; only the larger branch develops into a suspensor. This condition then rather approaches the formation of oöspores in Saprolegnia (compare *e*, Fig. 32, with Fig. 23). Zygorrhynchus is then an exception to the isogamy general in the Mucorales.

Zygorrhynchus moelleri (Fig. 32) is one of the most important of the Phycomycetes found in the soil. It is particularly abundant in loose sandy soils, sometimes forming enough mycelium to bind the loose particles together. In subsoils it is frequently the only fungus present. Soils poor in organic matter seem then to be especially favorable for its growth. It is active in ammonification, but does not decompose cellulose.

Circinella.—In Circinella the sporangia are formed only on lateral branches; not at the tips of the sporangiophores. These lateral branches frequently arise in whorls, six or eight branches arising from one point at regular intervals along the aerial filaments of mycelium. These lateral branches are also peculiarly curved upon themselves, a condition which is also found in *Mucor circinelloides* (Fig. 36). Species of Circinella are frequently found on the droppings of various animals.

Differentiation of Mucor and Rhizopus.—Mucor and Rhizopus may be readily differentiated from each other. Because of the runners, or stolons, Rhizopus tends to rapidly cover the surface of the agar plates, to climb the sides of the Petri dishes and fill the latter with mycelium. The rhizoids, or holdfasts, attach themselves to the under sides of the lids, frequently with sufficient firmness to require a little effort in removing the lids. While Mucors may fill up the Petri dish with mycelium, they do not thus attach themselves to the lid.

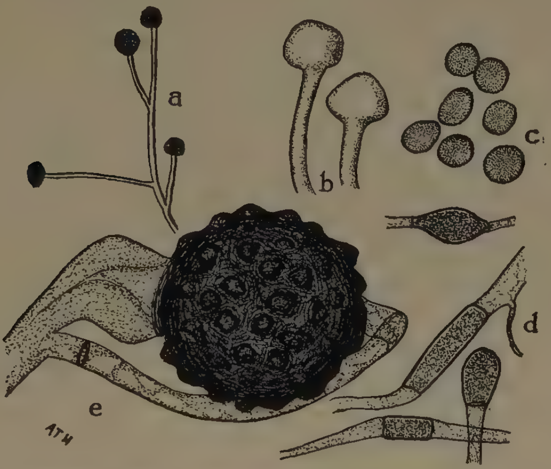

FIG. 32. *Zygorrhynchus moelleri.* *a.* Sporangiophore. *b.* Columellae. *c.* Spores. *d.* Chlamydospores in submersed mycelium. *e.* Zygospore.

The rhizoids or holdfasts of Rhizopus may be readily seen by focusing through the lid of the Petri dish with the low-power lens. The sporangiophores of Rhizopus arise from the nodes of the runners, i.e., at the point where the holdfast or rhizoids are formed. In Mucor the sporangiophores are formed from all parts of the plant. In Mucor the columella is always either round, cylindrical, or pear-shaped, never hemispherical, and is continuous with the sporangiophore. In Rhizopus it is hemispherical and rests in a cup-shaped expansion of the sporangiophore called the apophysis.

In Mucor, the spores though varying in form are always smooth and regular; in Rhizopus they are frequently angular, due to the fact that they readily collapse when mounted in water. The sporangiophores of species of Mucor grown in the light frequently show a positive phototropism, all of the sporangiophores bending towards the light. This does not occur with Rhizopus.

Species of Mucor.—There are many species of Mucor and their identification is not easy. Over fifty species have been described by Lendner [7] in his monograph. He considers as important characters for classification the occurrence of branching of the sporangiophores, their height and thickness, the diameter of the sporangium, the length and thickness of the columella, the dimensions of the spores, the degree of diffluence of the sporangial membrane, the form of the columella, and the shape of the spores. Since zygospores are so rarely formed in cultures, they cannot usually be used in diagnosis. Many of the characters given above are obviously subject to considerable variation even in a single individual, and cannot be relied upon too much.

The occurrence of branching of the sporangiophore is the most important single character. It cannot be easily determined in many cultures because of the close intertwining of the hyphae. The developing sporangiophores are frequently unbranched, therefore one cannot rely upon observations made at the edge of the growing colony where the mycelium is not so dense. Branching is best observed in slide cultures of the type shown in Fig. 25. A binocular dissecting microscope is of great value.

Three main types of branching are recognized: the Monomucors with unbranched sporangiophores, as in Fig. 33; the Racemomucors, with racemosely branched sporangiophores (i.e., a main stem with lateral branches) as in Fig. 34; and the Cymomucors, with sporangiophores typically branched as in *Mucor circinelloides* (Fig. 36) but frequently rather irregularly branched, as in *Mucor corymbifer* (Fig. 37).

KEY TO THE SPECIES OF MUCOR (LENDNER)[7]

Monomucors

A. Sporangiophores at first erect, later wilting to form a felt-like mass of rusty color. *M. rufescens.*

B. Sporangiophores always erect, forming a "turf."

- 1. Sporangiophores less than 2 cm. high.
  - a. Sporangiophores not more than 300μ high.
    - aa. On solid media, short velvety aerial mycelium, color at first reddish brown, later gray, sporangia 20μ (maximum). *M. Ramannianus.*
    - bb. Aerial mycelium hardly visible, sporangiophores 210μ, colorless, septate; sporangia 40–45μ diameter. *M. subtilissimus.*
  - b. Sporangiophores more than 0.5 cm. (maximum 2 cm.).
    - aa. Membrane of the sporangium not diffluent, breaking easily and leaving an irregularly torn collar, sporangia 36–42μ, spores elliptical, 8 × 6μ. Aerial mycelium 1.5 cm. high. *M. hygrophilus.*
    - bb. Membrane not diffluent, sporangia large (80–98μ), spores elliptical, 8 × 5μ. *M. adventitius.*
      Columella reddish orange. *var. aurantiaca.*

- 2. Sporangiophores more than 2 cm.
  - a. Spores mixed with droplets of oil and with granular protoplasm. *M. plasmaticus.*
  - b. No droplets of oil in the sporangium.
    - aa. Sporangiophores 2–3 cm.
      - I. Sporangia 80μ in diameter, columella oval, spores 10 × 8μ. *M. hiemalis.*
      - II. Sporangia larger (250–350μ), columella large, pear-shaped, spores 5–13μ × 6–12μ. *M. piriformis.*
    - bb. Sporangiophores more than 3 cm.
      - I. Membrane of the sporangium dissolving rapidly, columella often yellowish, spores 3–6μ × 6–12μ. *M. mucedo.*
      - II. Membrane dissolving slowly, columella colorless, spores very large, 15μ × 30–33μ. *M. mucilagineus.*

Racemomucors

A. Secondary branches verticillate, these in turn bearing verticillate branches. *M. glomerula.*

B. Branching frankly racemose or in corymbs.

- 1. Columella hemispherical, covered with colorless hairs resembling the capillitium of certain Myxomycetes. *M. comatus.*

2. Columella round, or oval, not as above.
   - a. Sporangiophores at first erect, then curving towards the substrate and wilting. *M. de Baryanus.*
   - b. Sporangiophores always erect.
     - aa. Species parasitic for other Mucors. *M. parasiticus.*
     - bb. Species not parasitic.
       - I. Sporangiophores of two sorts, one kind terminated by large sporangia with diffluent membranes; the others, lateral, carry sporangiola with persistent membrances. *M. agglomeratus.*
       - II. Species not possessing this character.
         - a′. Spores very unequal (a mixture of numerous small spores with others of double the size).
           - a″. Sporangiophores 0.5–1.0 cm., erect. Sporangia 80–125μ in diameter, spores round or angular, 4–15μ in diam. *M. heterosporus.*
           - b″. Sporangiophores ordinarily 3–4 mm. (1 cm. at the maximum), sporangia 70μ diam. (maximum). Spores oval or sub-cylindric, 2–6μ × 6–8μ. Giant chlamydospores along the course of the sporangiophore. *M. sylvaticus.*
           - c″. Sporangiophores 1 cm. Sporangia 40–54μ. Membrane fracturing. *M. lausannensis.*
         - b′. Spores equal in size.
           - a″. Sporangial membrane not diffluent, but rupturing into pieces.
             - AA. Spores round, 7μ in diam. *M. corymbosus.*
             - BB. Spores oval.
               - 1′. Sporangiophores often not branched, chlamydospores with very fine prickles, species forming numerous azygospores. *M. tenuis.*
               - 2′. Sporangiophores branched, chlamydospores with smooth walls, both zygospores and azygospores formed. *M. racemosus.*
           - b″. Sporangial membrane diffluent.
             - AA. Spores round, 3–3.5μ. *M. pusillus.*
             - BB. Spores oval or elongated.
               - 1′. Very large species, 6–8 cm. high, always more than 2 cm.
                 - 1″. Sporangiophores 6–7 cm. high, sporangia 300–400μ in diam., spores 7.5 × 17.5μ. *M. proliferus.*

- 2″. Sporangiophores 6–8 cm., sporangia 140–160μ, spores 9–12μ × 4.2μ. *M. flavus.*
- 2′. Smaller species, not more than 2 cm.
  - 1″. Columella largely subjacent to and concrescent with the membrane of the sporangium, diameter 100μ, spores 4–2μ. *M. mollis.*
  - 2″. Columella free or slightly flattened at the base.
    - A′. Spores small, oval, 4.2 × 2.1μ, bluish gray. *M. fragilis.*
    - B′. Spores elongated, convex surfaces unequal, 2–5μ × 5–10μ.
      - A″. Sporangia not more than 80μ, zygospores frequent, forming special branchings (on bread). *M. genevensis.*
      - B″. Sporangia 80μ on the average but may be 120μ in diameter, suspensors carry sporangiophores as with *M. racemosus*. *M. erectus.*

Cymomucors

A. Sporangiophores of two sorts, one erect and carrying normal spherical sporangia; the others spreading, circinate, sympodially branched and carrying pear-shaped sporangia. *M. pirelloides.*

B. Sporangiophores of one kind only.

- 1. Sporangiophores circinate (curved).
  - a. Sporangiophores hardly more than 1 cm., spores oval, 6μ in length at most.
    - aa. Sporangial membrane brown, sporangia often subsessile, spores 3–4μ × 5–6μ. *M. circinelloides.*
    - bb. Sporangial membrane bluish-black, sporangia carried on long, often circinate pedicels, spores 5–6μ × 4μ. *M. griseo-cyaneus.*
  - b. Sporangiophores from 1 cm. to 3 cm., spores round and 10μ or more.
    - aa. Sporangiophores spreading, 0.5–2.0 cm., sporangia black, 120–200μ, spores 10.5μ to 14μ in diam. *M. angariensis.*
    - bb. Sporangiophores erect, not circinate; other shorter ones much branched and circinate; sporangia smaller, 60μ on the average, but not more than 90μ, spores 10–12μ. *M. lamprosporus.*

2. Sporangiophores straight, not circinate.

a. Spores round and very unequal, of bizarre form. *M. heterosporus sibericus.*

b. Spores round and equal.

aa. Species growing poorly on must-gelatin, forming on bread aerial mycelium 2–3 mm. high, sporangia 50–70μ, spores 5–6μ. *M. jansseni.*

bb. Species growing well on must-gelatin and forming aerial mycelium 1–3 cm. high.

I. Columella spinous.

a′. Sporangiophores not more than 2 mm., sporangia 60–68μ, spores smooth, 7–8μ. *M. spinescens.*

b′. Sporangiophores 1 cm. or more, spores somewhat prickly, 5–8μ. *M. plumbeus.*

II. Columella smooth.

a′. Sporangia 75–120μ, columella pear-shaped or bell-shaped, spores 4–8μ. *M. globosus.*

b′. Sporangia ordinarily smaller (110μ, max.), columella round, oval, or bell-shaped. Spores larger, 10μ on the average. Species presenting sporangiola near the substrate.

a″. Sporangia 70–110μ, sporangiola not evanescent, spores round, brilliant, 10μ. *M. sphaerosporus.*

b″. Sporangia not more than 80–90μ, spores 10μ, sporangiola circinate, evanescent, sporangiophores higher than *M. sphaerosporus.* *M. lamprosporus.*

c″. Sporangia 60–80μ, normal spores 8–10μ, spherical, accompanied by abnormal spores, oval, 8–10μ × 30μ, no sporangiola. *M. dimorphosporus.*

c. Spores oval.

aa. Very large species, 9–12 cm. high.

I. Sporangiophores 9–10 cm., sporangia up to 1 mm. in diam., spores 10.5 × 28μ. *M. irkutensis.*

II. Sporangiophores 10–12 cm., sporangia 500μ, spores 8.6 × 5μ. *M. wosnessenskii.*

bb. Smaller species

I. Sporangial membrane not diffluent, breaking into pieces. *M. brevipes.*

II. Membranes of the first sporangia diffluent.

a′. Spores elongated, spore membrane prickly, sporangia black, 100μ. *M. ambiguus.*

b′. Spores subspherical with smooth membranes.

a″. Species forming a slightly elevated down on bread or must-gelatin, yellow. *M. rouxianus.*

- b″. Species forming a well-developed "turf" 1–3 cm. high.
  - AA. Sporangiophores but slightly branched, 1 or 2 lateral branches.
    - 1′. Sporangia 50–350μ, columella round, spores round, elliptical or angular, 4.2 × 6.5μ, chlamydospores. *M. geophilus.*
    - 2′. Sporangia 90–170μ, columella ovoid, spores subspherical, 5–6μ × 6–8μ, rarely 10μ. *M. strictus.*
  - BB. Sporangiophores much branched.
    - 1′. Sporangia 35–70μ (90μ, max.), spores 8 × 6μ, or 8–10μ in diam., yellow pigment in the hyphae, slightly developed. *M. prainii.*
    - 2′. Sporangia 50μ, membrane not diffluent, spores most often oval and smaller, 5–7μ × 4–5μ, also 4–7μ in diam. *M. javanicus.*

Mucor mucedo is perhaps the best-known species, being easily obtainable if fresh horse manure is incubated in a moist chamber. It has frequently been chosen as a type of the genus in physiological experiments. It is proteolytic and also capable of splitting fats. It is a cause of spoilage of various foodstuffs at times, is supposed to play a part in the ripening of snuff, may cause a decomposition of leather, and is found in retting flax. It may be recognized by the unbranched sporangiophore and cylindrical columella. No oidia or chlamydospores are formed. It is heterothallous.

Mucor hiemalis is very similar save that the columella is typically spherical (Fig. 33). It is concerned with the retting of hemp, secreting an enzyme which dissolves the middle lamella of the intercellular substance. It digests starch and gelatine, and ferments dextrose but not sucrose. It also is heterothallous.

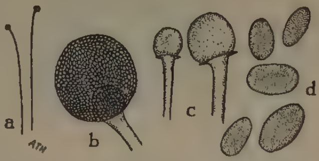

FIG. 33.—*Mucor hiemalis*. *a*. Sporangiophores. *b*. Sporangium. *c*. Columellae. *d*. Spores.

Mucor piriformis is recognized by its very large sporangia and pear-shaped columella. It is found on spoiled fruit and is claimed to be a cause of soft rot of pears.

Mucor Racemosus.—*Mucor racemosus* is the most common of the Racemomucors, and though of little practical importance is of some scientific interest. It is remarkable in that it produces an alcoholic fermentation of sugars, and that when submerged in sugar solutions the mycelium breaks up into a series of spherical oidia ("kugelzellen" in German literature) which may continue to grow as single cells by budding, like yeasts (Fig. 35). The association of the yeast-like form with alcoholic fermentation naturally attracted great interest.

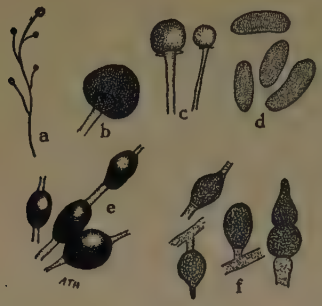

Fig. 34.—*Mucor racemosus*. *a*. Sporangiophore. *b*. Sporangium. *c*. Columellae. *d*. Spores. *e*. Chlamydospores on the aerial mycelium. *f*. Chlamydospores on the submersed mycelium.

Pasteur had maintained, in his theory of intramolecular respiration (which will be explained later in connection with the yeasts), that alcoholic fermentation takes place only in the absence of a sufficient oxygen supply, and it was found that the lack of oxygen is the factor which determines the production of the yeast-like oidia. It was then easy to assume that the yeast-like structure

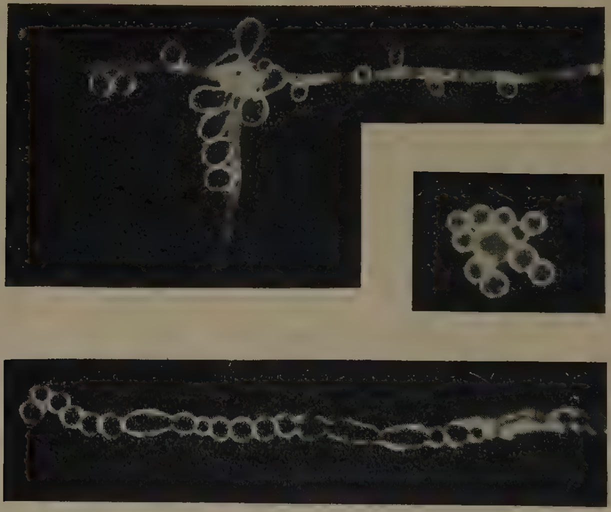

Fig. 35.—*Mucor racemosus*. Production of oidia by submersed mycelium, and multiplication of these by budding. Photomicrograph by dark-field illumination.

in fungi is necessarily correlated with alcoholic fermentation. This, however, was later proved to be not the case, for as much alcohol is formed by the Mucors under aerobic conditions as under anaerobic, yet the yeast-like forms are not produced. On the other hand, many yeasts have been found which do not form alcohol. It is curious, however, that these round oidia are formed only by Mucors which also produce alcohol. Their resemblance to yeasts is only superficial, as they are much larger, and each cell contains many nuclei.

Mucor racemosus is easily recognized by the characteristic branching of the sporangiophores. The sporangial wall is frequently covered with tiny crystals of calcium oxalate, and the columella is somewhat pear-shaped. Inoculation into dextrose broth fermentation tubes will show the production of gas and yield the spherical budding cells in the sediment. Perhaps the most striking character is the abundant formation of the jet-black chlamydospores in the aerial mycelium. *Mucor racemosus* may be distinguished from the closely related *Mucor erectus* and *Mucor fragilis* by the form of the columella. The latter two species also form slight amounts of alcohol from sugar and give rise to budding oidia in the submerged portions.

M. rouxianus.—Although the preceding species form but small amounts of alcohol, another Mucor, belonging to the Cymomucor group, *Mucor rouxianus* (sometimes called *Mucor rouxii*) has been used industrially for the production of alcohol. It secretes both diastase and zymase, and can therefore produce alcohol directly from starch without any malting process. It is used in the Orient for preparing alcoholic beverages from rice, and is marketed under the name "Chinese yeast," in little balls of rice meal, much as we sell yeast cakes. It is also used commercially for alcohol production in Europe.[1] More than 5 per cent of alcohol is produced by the fermentation.

This species may be recognized by the characteristic branching of the sporangiophores and the spherical columella with large oval spores. In the submerged mycelium, large irregular thick-walled cells may be formed. Like the Racemomucors mentioned above, black chlamydospores appear in the aerial mycelium, and budding yeast-like cells in submerged portions. Abundant fat globules may develop in the mycelium, especially on starchy substrates; these take on a deep yellow color.

There are several other species related to *Mucor rouxianus* which have been isolated from other Oriental "yeasts," as *Mucor prainii* from India, and *Mucor javanicus* from Java.

Some authors have grouped together those Mucors which tend to break up into round oidia, or to produce numerous chlamydospores, in a separate genus, Chlamydomucor, but these structures are found in so many different kinds of molds that they should not be given much weight in classification.

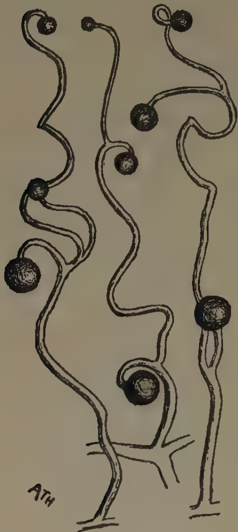

Fig. 36.—*Mucor circinelloides*, sporangiophores showing typical cymose branching.

Mucor circinelloides is frequently encountered in soil cultures. It exhibits true cymose branching (Fig. 36). Budding oidia are formed in mycelium submersed in liquid media. *Mucor plumbeus* is also a common soil form. It derives its name from the lead-gray color of the mycelium. It may be identified by the peculiar spiny columella and the prickles on the spores.

Pathogenic Mucors.—A series of pathogenic phycomycetous molds have been encountered occasionally in man and animals. This group contains species (apparently somewhat variable) which appear to occupy a position transitional between the genera Mucor and Rhizopus. Some have been described as species of Mucor, others as species of Rhizopus, while Vuillemin created a separate genus for some of them, named *Lichtheimia*. For literature and differential characters, see Sartory.[10]

The species found in man is generally referred to as *Mucor corymbifer*. It presents some peculiarities which serve to differentiate it from the other Mucors, notably the tendency of the sporangiophores to rest on the surface of the substrate rather than project into the air, and their characteristic branching in corymb-like clusters (Fig. 37). The columella varies in form, but tends toward the piriform; there is a funnel-shaped expansion of the sporangiophore beneath the columella, approaching the formation of an apophysis as in Rhizopus. According to Sartory, holdfasts may sometimes be formed. It is a curious fact that the patho-

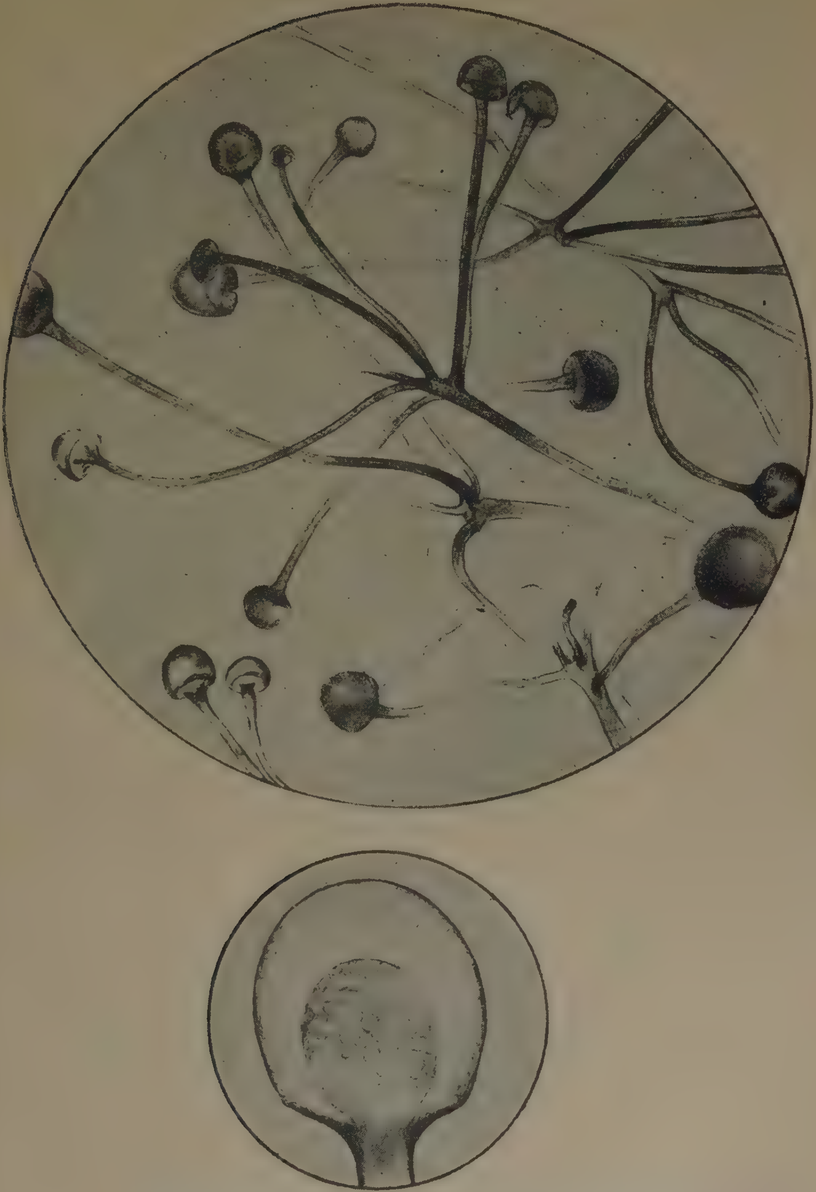

Fig. 37.—A pathogenic Mucor, probably *M. corymbifer*, isolated by Ernst.[4]

genicity of this species was first discovered by Lichtheim [8] by experimentally inoculating rabbits, its occurrence in natural infections being discovered subsequently.

The first clear-cut case of human infection, reported by Paltauf,[9] was one of generalized infection which began in the lungs, multiple abscesses developing in various parts of the body, with a fatal outcome. In the tissues the organism grows as a rather coarse mycelium. Only in the walls of the bronchi, where oxygen is abundant, are sporangia produced. Since this first case, several others have been reported, all primary in the lungs, including one (by Ernst) [4] in this country, while in some others the organism has been found in sputum where it may possibly indicate the presence of a secondary infection, as in tuberculosis. The majority of reported cases of infection have, however, been cases of simple external ear infection, and even here this fungus is rare as compared with other molds, being the etiologic agent in only about 4 per cent of the cases of otomycosis.

A species closely related to the above was described by Lichtheim as *Mucor rhizopodiformis*, but is now more generally known as *Rhizopus cohnii*. Like the former species, its pathogenicity was first determined by animal inoculation, but it has since been found as a cause of abortion in cattle, the mold being isolated from the fetal membranes from cases in which there was no evidence of *B. abortus* or other infecting agents. The mycelium is found invading the placenta, and also the respiratory passages of the fetus.[11]

KEY TO THE SPECIES OF RHIZOPUS (LENDNER)

A. Spores irregular, angular, subspherical, or oval.
  1. Spores striate.
    a. Sporangiophores erect.
      aa. Sporangiophores in groups, spores more than 4μ.
        I. Rhizoids well developed.
          a′. Rhizoids irregularly distributed on the stolons; sporangiophores branched. Pathogenic species not growing well save between 33° and 37° C. *R. parasiticus.*
          b′. Rhizoids normal, at the base of the cluster of sporangiophores.
            a″. Large species of the type of *R. nigricans.*
              AA. Not growing on potato at 39° C. *R. nigricans.*

BB. Growing on potato at 39° C.

1′. Growing very well between 37° and 39° C. *R. orizae.*

2′. Not growing so well.

1″. Not fermenting sucrose, melibiose, raffinose or inulin; fermenting trehalose. *R. tonkinensis.*

2″. Fermenting sucrose, melibiose, raffinose and inulin, not fermenting trehalose. *R. japonicus.*

Other closely related species.

Sporangia 85–210μ, spores 5–6μ, often irregular. *R. tritici.*

Sporangia 48–149μ, spores 6–8μ. *R. tamari.*

Sporangia 47–109μ, spores 4–8μ. *R. cambodia.*

II. Rhizoids poorly developed.

a′. Rhizoids rare, pale, short; spores oval or round, 5–7μ; Sporangiophores without nodosities. *R. arrhizus.*

b′. Rhizoids rare; spores oval, angular, irregularly polyhedric, 6–9μ; sporangiophores and stolons branched, swollen in places. *R. nodosus.*

bb. Sporangiophores single, small, spores not more than 4μ.

I. Sporangiophores 0.5–0.8 mm., spores 4μ. *R. microsporus.*

II. Sporangiophores not over 0.3 mm., spores 3μ. *R. minimus.*

b. Sporangiophores bending over.

aa. Sporangiophores in clusters, 2–2.5 mm., spores 8–10μ. *R. reflexus.*

bb. Sporangiophores single, 0.2 mm., spores 5–6μ. *R. circinans.*

2. Spores smooth.

a. Pathogenic species.

aa. Columella conical or subcylindric; fungus associated with "black tongue." *R. niger.*

bb. Columella ovoid or pear-shaped, fungus pathogenic for rabbits.

I. No chlamydospores. *R. cohnii.*

II. Chlamydospores. *R. equinus.*

b. Non-pathogenic species, found in leavens of the Far East.

aa. Spores 5–7μ, sporangia 70–80μ. *R. chinensis.*

bb. Spores 7–10μ, sporangia 180μ. *R. oligosporus.*

B. Spores round, not angular; smooth or spiny.

1. Spores spiny, 15μ in diameter. *R. echinatus.*

2. Spores smooth, 5–7μ; sporangia 50–70μ. *R. elegans.*

3. Spores smooth, 2–4μ; sporangia larger, 90–140μ. *R. speciosus.*

Rhizopus nigricans is by far the most common of all the molds belonging to the Phycomycetes. It is continuously encountered in all kinds of bacteriological work as an air contamination, and is particularly annoying because of its ability, by means of its stolons, to rapidly overgrow Petri plate cultures, covering the entire surface of the agar in twenty-four hours.

It is especially important as a cause of spoilage of fruits and stored potatoes, especially sweet potatoes. The small fruits, especially strawberries, are particularly susceptible. The disease in strawberries is known as "leak," due to the softening and dripping of the fruit. It occurs as a source of considerable loss in shipment and is prevented by refrigeration. In sweet potatoes, a characteristic soft rot is produced, the main factors determining which are injury to the potatoes and humidity of the storage bins. For a bibliography of the rots caused by *Rhizopus nigricans*, see Heald.[5]

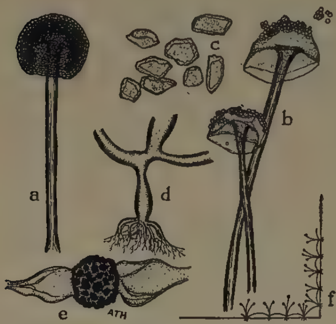

FIG. 38.—*Rhizopus nigricans*. *a*. Mature sporangium. *b*. Ruptured sporangia, showing the flattened columellae. Note the funnel-shaped expansion (apophysis) of the sporangiophore beneath the columella. *c*. Spores. *d*. A rhizoid, or holdfast. *e*. Zygospore. *f*. Diagram showing mode of growth by runners.

A number of species of Rhizopus closely resembling *Rhizopus nigricans* have been obtained from ferments used in Oriental countries. They are all very active in changing starch to sugar. *Rhizopus japonicus* occurs as a contaminant or accompanying ferment along with *Aspergillus oryzae*, in Japanese "koji," a commercial preparation of molds growing in rice meal, used in the preparation of soya sauce. It differs from other species of Rhizopus in the abundant production of chlamydospores throughout the mycelium. It is actively diastatic, and has been used commercially in Europe in malting corn meal preparatory to alcoholic fermentation. It also produces a little alcohol itself. *Rhizopus oryzae* is a similar mold obtained from Javanese "raji," a ferment used in the preparation of the alcoholic beverage "arrak" from rice. *Rhizopus tritici* is obtained from a preparation grown on wheat meal. Some five or six other species obtained from Oriental ferments have also been described. Several different species have also been described as causes of soft rots of various fruits and vegetables. These species are differentiated more on physiological than morphological characters.

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CHAPTER V

MOLDS BELONGING TO THE ASCOMYCETES AND THE FUNGI IMPERFECTI

Since ascospores are formed by very few of the molds likely to be encountered by the bacteriologist, and since those forms which do develop such sexual spores are closely related to numerous other forms which are imperfect (and are therefore generally grouped with them), for practical purposes the Ascomycetes and Fungi Imperfecti may be considered together.

The classification of the Fungi Imperfecti is very unsatisfactory. Undoubtedly in a large proportion of them the imperfection lies in our knowledge of their life-cycles rather than in the fungi themselves. Since the sexual forms are unknown, we must base our classification upon the non-sexual reproductive bodies or conidia which may be produced in a great variety of manners. But it must be kept in mind that these conidia do not, in all probability, represent any true phylogenetic relationship; for species which had previously been classed in the same genus according to the formation of their conidia, were discovered (after their sexual forms were found) to belong to three different classes, namely Phycomycetes, Ascomycetes and Basidiomycetes! That is to say, widely diverse fungi may form non-sexual spores in precisely the same manner. Any classification of the Fungi Imperfecti must therefore be considered an artificial one for practical purposes and not a natural one indicating systematic relationships.

The problem is complicated by the fact that in many cases it is somewhat difficult to decide just what are the conidia. As was pointed out in the first chapter, many fungi, in addition to their spores, multiply by the formation of yeast-like cells either by fragmentation of the mycelium or by budding. These are not generally looked upon as specific characters. They are differentiated from conidia by the fact that the latter do not grow at once if separated from the parent mycelium. But one may find all transitions (in some species) between these oidia and the true conidia. So it happens that the same organism may be classified in one genus by one authority, and in an entirely different genus by some other writer, due to different interpretations of the same structure; for instance, the organism of thrush, now generally considered as a Monilia, has been placed in half a dozen or more genera. Moreover, the same fungus may form more than one type of conidia, or may produce its conidia on more than one type of fruiting body. Thus the organism of soft rot in apples forms conidiophores scattered over the surface of the colony on artificial media, and because of the type of branching it is placed in the genus Penicillium; but on apples these same conidiophores are formed in dense clusters, called coremia, and upon that basis the organism would be included in the genus Coremium.

The classification of the Fungi Imperfecti followed by most mycologists, and in general use in this country, is based upon the system proposed by Saccardo. But another, quite different, classification has been introduced by Vuillemin, which is in quite general use by French mycologists; and since so large a proportion of the literature on medical mycology has come from France, this system cannot be ignored by the bacteriologist.

Vuillemin's Classification.—Vuillemin divided the Fungi Imperfecti into three orders[50] based upon the characters of the reproductive bodies. Later a fourth order, the Microsiphonales, was added,[51] to include the organisms generally referred to as Actinomycetes; but more recently these have been reduced to the rank of a family.[52] Vuillemin's classification is based upon new definitions of the reproductive bodies, as follows:

Thallospores are cells formed by the vegetative mycelium, more vegetative in character than adapted for disseminating and conserving the species. Two types are recognized, the blastospores and the arthrospores. *Blastospores* are rounded cells, resembling yeasts, produced by budding from the tips or along the sides of the mycelium, and by continued budding giving rise to a series of similar cells; *arthrospores* are produced by disarticulation of the mycelium itself. *Hemispores* are structures apparently transitional between thallospores and conidia; certain cells of the mycelium, without developing typically into conidiophores, develop a swollen structure which later breaks up into a series of spores. *Aleuriospores* (in French, "aleuries") are differentiated from true conidia by the fact that they are not set free when mature, but are only liberated on the disintegration of the mycelium that forms them; they are analogous to lateral chlamydospores, and are especially characteristic of the ringworm fungi. *Phialides* are bottle-shaped stalks upon which conidia are formed, i.e., the sterigmata of such molds as Aspergillus and Penicillium.

CLASSIFICATION OF THE FUNGI IMPERFECTI ACCORDING TO VUILLEMIN

Order *Thallosporales*, reproducing by thallospores.

- Suborder *Blastosporineae*, reproducing by blastospores. This group includes the non-spore-forming yeasts and fungi with yeast-like forms, as Monilia.
- Suborder *Arthrosporineae*, reproducing by arthrospores. This group includes such forms as *Oidium lactis*, and according to the recent rearrangement, the Actinomycetes. It comprises two families.
  - Family *Mycodermaceae*, with "normal" septate mycelium.
  - Family *Nocardiaceae*, with non-septate mycelium, very fine, of bacterial dimensions (the Actinomycetes).

Order *Hemisporales*, reproducing by hemispores.

This order contains but one unimportant genus, Hemispora.

Order *Conidiosporales*, reproducing by conidia.

- Suborder *Aleuriosporineae*, reproducing by imperfect conidia or aleuriospores. The ringworm fungi would belong here according to Ota and Langeron.
- Suborder *Sporotrichineae*, reproducing by true conidia, which are not borne upon conidiophores, as in Sporotrichum.
- Suborder *Sporophorineae*, reproducing by true conidia borne upon true conidiophores. This group would contain most of the common molds of the Fungi Imperfecti, save those of the next suborder.
- Suborder *Phialidineae*, reproducing by true conidia borne upon phialides (or sterigmata), including Aspergillus and Penicillium.

Saccardo's Classification.—Saccardo's arrangement makes a primary subdivision into three orders upon the following basis: Certain of the fungi parasitic on plants form conidiophores within a globular mass of protecting mycelium, called a pycnidium, from which they are discharged when mature through an opening on the surface of the plant tissue; these forms are placed together in an order known as the *Sphaeropsidales*. Other plant pathogens produce conidiophores closely packed together, from the surface of a flat, plate-like mass of pseudoparenchyma on the surface of the host plant, known as an acervulus; such species comprise the order *Melanconiales.* The third order, the *Hyphomycetales* (frequently called Moniliales), contains the remaining forms, whose conidiophores are produced neither in pycnidia nor upon acervuli, but are formed from superficial hyphae over the entire surface of the fungus colony. Those molds of interest to the bacteriologist will, of course, all be found in this last order.

The Hyphomycetales are further subdivided into groups which have in some works been given the rank of families, in others, of suborders. Two of these are based upon a characteristic grouping or bunching of the conidiophores. In the *Stilbaceae* the conidiophores are clustered to form characteristic stalked bodies of cylindrical form, the coremia. In the *Tuberculariaceae* the clusters of conidiophores form globose bodies without stalks, the sporodochia. Neither of these families contains species important to the bacteriologist, save that coremium-forming Penicillia have sometimes been included with the Stilbaceae.

The remaining Hyphomycetales are grouped into two families according to the color of the mycelium. If this is white or brightly colored, they are included in the *Mucedinaceae*; if it is dark, smoky (black or shades of brown or olive), they belong to the *Dematiaceae.* Since the color of the conidia is frequently quite different from that of the mycelium, the color of the latter is best determined from the reverse side of the colony.

A very large number of genera and species of Hyphomycetales have been described. A very large proportion of these are quite rare, many of them are of doubtful validity. To present a complete key would defeat the purpose of this book. But experience shows that the molds of importance to the bacteriologist all fall within a very few genera; fully three-fourths of those encountered in routine work will be species of Aspergillus and Penicillium. The author therefore proposes to present only a very simplified key to those groups which he has found frequently in bacteriological work.

The key to the Hyphomycetes published by Lindau in Rabenhorst's Kryptogamenflora has been followed by most mycologists. It is reprinted in Waksman's "Principles of Soil Microbiology" [53] and in Buchanan's "Bacteriology." [6] Very complete keys with descriptions and illustrations of species found in soil will be found in the article by Gilman and Abbott.[19] Keys to species pathogenic to man, based upon Vuillemin's classification, are presented in Castellani's "Fungi and Fungous Diseases,"[8] and in Sartory's "Champignons Parasites."[38] Forms which are plant parasites (many of which also lead a saprophytic existence) may be identified by reference to Stevens' "Plant Disease Fungi."[42] The reader is referred to these works for aid in identifying molds which are not included here.

The key below does not include the ringworm fungi, whose position is uncertain, and which require special treatment. They will be considered in the next chapter.

KEY TO SOME COMMON OR IMPORTANT HYPHOMYCETALES

A. Mycelium white or brightly colored. (Mucedinaceae.)
    1. Conidia one-celled.
        a. Conidia not borne on definite conidiophores.
            aa. Conidia not easily differentiated from oidia. Reproduction mainly by the latter. These are forms transitional between molds and yeasts (see Chap. VII). *OIDIUM* or *MONILIA*.
            bb. Conidia borne laterally or terminally from all parts of the mycelium. *SPOROTRICHUM*.
        b. Conidia formed on definite conidiophores, in heads.
            aa. Conidia in clusters, not chains. *TRICHODERMA*.
            bb. Conidia in chains.
                I. Conidiophores unbranched, arising from a foot cell. Conidia formed by sterigmata arising from the inflated end of the conidiophore. *ASPERGILLUS*.
                II. Conidiophores branches at the tip.
                    a′. Conidia thin-walled, without a ring or collar at the base. *PENICILLIUM*.
                    b′. Conidia thick-walled, with a ring or collar at the base. *SCOPULARIOPSIS*.
    2. Conidia two-celled. *CEPHALOTHECIUM*.

B. Mycelium dark or smoky. (Dematiaceae.)
    1. Conidia one-celled. Conidiophores much branched at the tip, and forming branched chains of conidia. *HORMODENDRUM*.
    2. Conidia two-celled, otherwise as above. *CLADOSPORIUM*.
    3. Conidia many-celled, muriform, in chains. *ALTERNARIA*.

Sporotrichum and Sporotrichosis.—The genus Sporotrichum is characterized by the production of pear-shaped conidia directly from the mycelium, conidia arising both laterally and at the tips of the filaments, from all parts of the mycelium. A large number of species is known, mostly saprophytes on vegetable matter; at least one is a plant pathogen, and one, perhaps several, cause disease in man and animals. Sporotrichosis is one of the most important of the mycoses, perhaps the most common of the serious fungous diseases.

Geographic Distribution.—The disease, first recognized in this country by Schenck, and shortly afterwards in France by de Beurmann, has since been found in all parts of the world. But the great majority of the reported cases have occurred in France and the United States; a number also from South America. There is some evidence of a limited geographical distribution. Thus Ruediger [37] found five-sixths of the cases reported in America by 1912 had occurred in the valley of the Missouri River. According to Foerster (1926),[17] 130 out of 148 cases reported in the United States were in the valleys of the Mississippi or its tributaries, and a large proportion of these are in the Missouri valley. Meyer [26] also found that outside of Pennsylvania most of the cases of equine sporotrichosis occurred in the Missouri valley.

This geographical distribution may be due to a greater prevalence of the parasite in this region, perhaps parasitic upon some indigenous plant, or to a larger proportion of the population (agricultural) engaged in occupations which expose them to infection; or (and this seems the more likely) to the fact that the medical profession in these districts have been on the lookout for such cases. In recent years more and more cases have been reported from other parts of the world. This increasing number of cases reported is also probably due to an increased alertness of the medical profession rather than an actually greater prevalence of the disease.

Sources of Infection.—The infection may occur in various ways, but in a large proportion of cases it is clear that the parasite has been introduced into the tissues from or on vegetable matter of one sort or another. The organism has been found growing free in nature upon a grain by Sartory; and from the histories of numerous cases, we must assume that it is a fairly widespread saprophyte upon all sorts of vegetable matter. Thus Foerster [17] noted that 14 of his 18 cases followed wounds of the upper extremity by barberry thorns. Most of the cases, however, where the source of the infection could be traced, have been due to wounds caused by hard masses of vegetable matter, as straw or grains. A very large proportion of the cases have occurred in farmers, in some cases following wounds caused by agricultural implements. In many cases it has been impossible to determine the source of infection.

The disease also occurs spontaneously in certain of the lower animals, notably horses and rats. A number of human cases have been contracted either directly (by bites) or indirectly from such lower animals.[26, 28] There have been two accidental laboratory infections, one from an equine strain, the other from a culture of human origin. In at least one case there has been direct transmission from man to man.

Portals of Entry.—There are two portals of entry, through wounds and through the alimentary tract. The great majority of human cases have been primarily wound infections, but a few cases presenting a generalized infection without any primary focus have been interpreted as an invasion through the mucous membrane of the intestinal tract. Davis was thus able to produce a generalized infection in rats by feeding the organism. A few cases have been reported in which apparently a primary involvement of the lungs occurred. But in most of the cases, the lesions will be seen in the skin and subcutaneous tissues. Foerster reports that 111 out of 146 cases were primary on the hands.

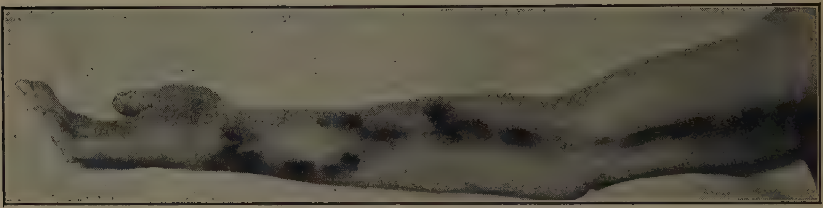

Fig. 39.—Sporotrichosis.

The clinical picture of a typical case is so striking that, once seen, the disease will always be readily recognized. There will be seen extending in a line upon the surface of an extremity, a series of hard, elevated, reddened lumps, the older lesions presenting a fistula from which pus may be expressed. At first glance the lesions look like boils but are not hot and tender, and there is practically no constitutional reaction. The firmness of the nodules suggests a syphilitic gumma. Between the lesions the course of the subcutaneous lymph vessels can frequently be traced as reddened lines. Although in the majority of cases the infection spreads from the primary lesion by way of the lymph vessels, it seldom goes beyond the regional lymph nodes. Cases of generalized infection by way of the bloodstream occur, but are relatively rare. Metastatic lesions may occur in the lungs, liver, and especially frequently in the testicles. One gains the impression that such generalized cases are more frequent in Europe than in America. In some cases the disease may be transmitted from the arm to some other part of the skin surface by contact.

Diagnosis.—The disease may present so close a resemblance to tertiary syphilis that it has undoubtedly been frequently misdiagnosed for that disease. An incorrect diagnosis subjects the patient needlessly to a prolonged course of treatment, without any benefit unless iodides are administered. A correct diagnosis with proper treatment (internal administration of iodides) leads to a prompt and permanent cure. Since iodides are commonly used in the treatment of tertiary syphilis, one may make an erroneous diagnosis of syphilis in a case of sporotrichosis, and believe that the diagnosis has been confirmed by the results of specific treatment.

The diagnosis is established by demonstrating the organism in the pus. In the body tissues and exudates the parasite appears as a small, single-celled, spindle-shaped organism. It is Gram-positive and is most frequently seen within the polymorphonuclear leucocytes. It apparently reproduces by fission. In size the cells are comparable to those of some of the larger bacteria, but are easily recognized by their characteristic cigar shape. They have sometimes been incorrectly referred to as spores. They are quite different from the true spores which are formed in cultures. They are the only structures formed in the body, no mycelium develops. They are far from being numerous in pus from human cases, and may be found only after prolonged search. They are best looked for in smears stained by Gram's method.

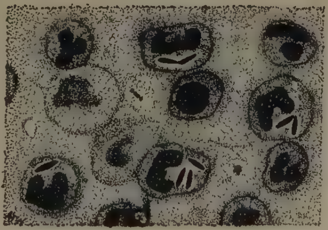

FIG. 40.—Smear of pus from sporotrichosis, showing the parasites within the leucocytes.

Although these bacillus-like cells are never found in ordinary cultures, according to Davis[13] they are formed in cultures in blood or blood serum, especially if air is excluded or if sterile tissue is added.

Cultures.—Cultures are of more value in diagnosis than are smears. Pure cultures may be readily obtained if made from pus aspirated from the younger lesions which have not yet opened; with more difficulty from those which have developed fistulae, for in these latter there is always considerable secondary infection with bacteria. The fungus will not grow readily on dextrose-tartaric agar, and the medium of choice is Sabouraud's agar.

The character of the growth on agar is strikingly different from that of most molds. At first it is soft and creamy in texture, the surface moist and shiny, whitish in color, and resembles more a culture of bacteria. As the culture grows older, it becomes darker in color, first a light tan which gradually deepens to a coffee brown and may eventually become quite black. The mass becomes firmer in texture, tending to pull off the agar in rather elastic flakes, and the surface becomes more and more wrinkled. But the surface nearly always remains rather shiny, not developing the cottony masses of aerial mycelium which is found with most molds. Occasionally a strain will develop some aerial mycelium, usually in the form of small prickle-like excrescences on the surface composed of an aggregation of filaments. Abundant aerial mycelium may develop on prune extract agar.

If some of this growth is examined under a microscope there is found a tangled mass of branched mycelium and a large number of free pear-shaped conidia. The latter are always freed from the mycelium when such a mount is prepared. To see their normal relationship it is necessary to prepare slide cultures. This was done by de Beurmann and Gougerot[3] by placing sterile slides in wide test-tubes with a small amount of dextrose broth into which the organism was inoculated. As it grew, the mold would climb the slide for a short distance, and if this were removed and fixed, the arrangement of the parts could be readily observed. In the author's experience, however, the mold will grow for only a millimeter or two up the slide, and is very apt to slip off when the latter is removed from the tube. The method of agar slide cultures described in Chapter II is preferable. In such a preparation the spores can be seen to be given off from all parts of the mycelium, both laterally and at the tips, sometimes singly and sometimes in small clusters. Sometimes the spores appear to be attached to short stalks. These, however, are part of the spore. When the spores break loose, the pedicel goes with them. In slides which have been dehydrated and mounted in balsam, these pedicels appear much more slender than in wet preparations (Fig. 41). Chlamydospores may also develop in the course of the mycelium. These on germinating may give rise to new round cells by budding. Such yeast-like cells are, however, rare.

Both cultural and morphological characters are subject to considerable variation. Of the former, the degree of pigmentation is most variable. Some strains may never develop more than a light tan color, others may darken very rapidly. The same strain may form more pigment on some media than on others, or on the same medium may remain colorless at one time and become pigmented in a later transfer. An inoculation of a pure culture on agar may show pigmentation in one portion of the growth and not in another.

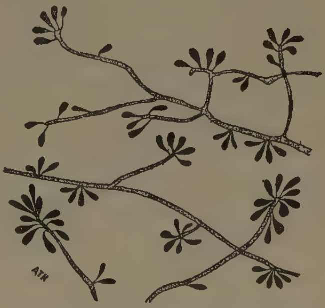

FIG. 41.—*Sporotrichum schenckii-beurmanni*. Stained slide culture preparation.

Species.—On the basis of these rather variable morphological and cultural characters a number of different species of pathogenic Sporotricha have been described. Buschke and Langer [7] recognize thirteen. It would seem, however, that the differences upon which these species have been made are within the limits of variability of a single strain. Greatest stress has been placed upon the differentiation between the American species, *Sporotrichum schenckii*, and the more common European type, *Sporotrichum beurmanni*. These have been separated mainly on the ground of pigment formation, *S. schenckii* being light and *S. beurmanni* dark; on the degree of spore formation, *S. schenckii* forming fewer lateral conidia; and on sugar fermentations, *S. schenckii* producing acid from lactose, not sucrose, while *S. beurmanni* is said to ferment sucrose but not lactose. But Davis has shown that both pigment formation [15] and spore formation [14] are too variable to be used for differentiation, depending more on the nature of the medium and rapidity of transfer than on the strain. And Meyer [25] has similarly found that sugar fermentations are highly variable. It would seem, therefore, that while different strains may appear somewhat unlike, there is no valid reason for recognizing more than one species, sometimes referred to as *S. schenckii-beurmanni.* Wolbach [55] has described another isolated from an infected knee joint which may well be a separate species. It produces an abundant aerial mycelium and no lateral conidia at all. He named it *Sporotrichum councilmanni.*

While various laboratory animals are susceptible to infection, rats are particularly so. In addition to making cultures, inoculation into a male white rat is a procedure of considerable diagnostic value. The inoculation should be made into the peritoneal cavity. There occurs a generalized peritonitis with minute nodules on all of the peritoneal surfaces, and in addition a very pronounced inflammation of the testicle which may be determined without sacrificing the rat. Unlike the human lesions, those in the rat contain the typical cigar-shaped cells in great abundance, and may be readily found in Gram-stained smears.

The agglutination reaction with spores of Sporotrichum has been discussed in Chapter III. This reaction, introduced by Widal and his co-workers, is of considerable diagnostic value; though cross reactions occur with thrush and actinomycosis, these diseases are not likely to be confused with sporotrichosis. Davis [12] was unable to differentiate various strains of Sporotrichum, including some of equine origin, by means of agglutination reactions. Cutaneous reactions are positive but are considered less specific than agglutination. Moore and Davis [28] got no reaction with an extract of the organism of blastomycosis in a case of sporotrichosis.

Trichoderma.—Not infrequently one finds a mold growing on plate cultures which is of a very bright pure green color; the aerial mycelium is very abundant, and frequently little tufts of white (sterile) aerial mycelium project above the conidiophores. Microscopic examination reveals numerous small clusters of conidia attached directly to the tips of the many-branched conidiophores (Fig. 42).

This is *Trichoderma köningi,* one of the most numerous of the soil fungi, and a very common contaminant in bacteriological work. It is the most active of the soil fungi in ammonification, liberating 57 per cent of the nitrogen in dried blood as ammonia within twelve days in one experiment. It is also very active in decomposing cellulose. On the other hand, it has no diastatic action at all.

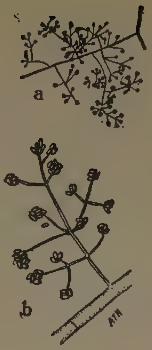

Fig. 42.—*Trichoderma köningi*. *a*. arrangement of conidiophores. *b*. higher magnification, showing clusters of conidia.

Aspergillus.—This very important genus contains a large portion of the molds which will be encountered in routine bacteriological work.

The genus may be recognized by the very characteristic arrangement of the conidia and conidiophores. The unbranched conidiophore arises from an enlarged cell of the vegetative mycelium, the foot cell, and terminates in a swollen portion, the vesicle. From the latter there are given off a number of little stalks, or sterigmata, having a characteristic tenpin form, which in turn bear the chains of conidia. This arrangement presents the spores in a compact mass or "head" at the tip of the hypha. In some species each sterigma is branched, giving rise to two or more secondary sterigmata. Such species have been placed in a separate genus, Sterigmatocystis, by some authors, but are usually included with Aspergillus. The structure and arrangement of a conidiophore are shown in Fig. 43.

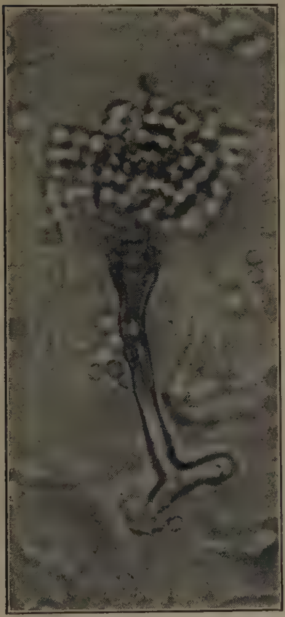

Fig. 43.—A conidiophore of *Aspergillus nidulans*, showing the foot-cell, stalk, vesicle, and chains of conidia. Photomicrograph from a slide culture

In some species of Aspergillus ascospores are formed, i.e., they are perfect fungi and should be included with the Ascomycetes. Some authors make a separate genus of these forms, called Eurotium, placed in the Ascomycetes. Of the common species ascospores are formed most readily by *A. glaucus* and *A. nidulans*. The perithecia develop from peculiarly coiled hyphae. When the perithecia are mature, they appear on the surface of the colony (in *A.*

glaucus) as small yellow dots easily visible to the naked eye (Fig. 44). In some cultures they may be developed in large numbers. The wall of the perithecium is very firm and composed of polygonal cells. The ascospores, eight in number, are contained in clear oval asci, surrounded by much loose cellular tissue (Fig. 45). They may be seen by crushing a perithecium under a coverglass. In some cases the asci may be very few in number, and not infrequently one finds bodies having the general appearance of perithecia but containing no asci. These are known as sclerotia. In some species only such sterile sclerotia have been found, no asci being known. In still others neither sclerotia nor perithecia have been seen.

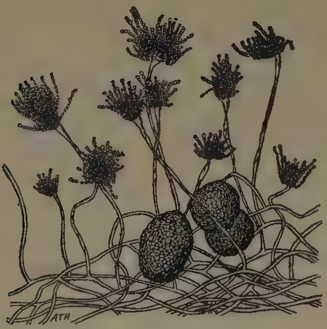

Fig. 44.—*Aspergillus glaucus*, showing arrangement of conidiophores and perithecia.

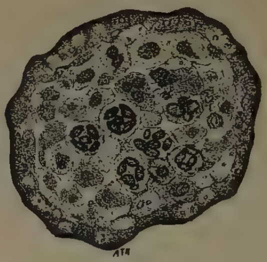

Fig. 45.—Section through a perithecium of *Aspergillus glaucus*, showing development of asci and ascospores.

The various species are identified to great extent by the color of the spores, which is fairly constant. In making use of this color, however, it is important to examine the culture at the right stage of growth. When too young the spores have not developed sufficiently, and the color of the surface of the colony is whitish; on the other hand, after the culture has aged the color becomes darker and less definite, gray or olive or brown. Frequently the vegetative mycelium develops a different color, best observed from the bottom of the culture dish, usually yellowish or reddish. This is not so constant or characteristic as the color of the spores, varying more with the composition of the medium.

A number of species are so nearly alike in color that final identification must always be based upon microscopic examination, especially of the spore heads. The characters to be observed are the size and form of the vesicle, the number and arrangement of the sterigmata, the presence or absence of secondary sterigmata, and the size and form of the conidia. All of these characters together tend to give the spore head itself a characteristic appearance. The spores tend to be arranged in a dense mass which may be globular, oval or cylindrical in form. The height of the conidiophores is also of some importance. Where present, the position and color of the perithecia and the form of the ascospores are important. But they are produced very infrequently by most varieties.

Thom and Church[47] recognize sixty-six valid species, and a number of others have been named. However, a large number of these are rare and of no great importance. For a complete key to the species as well as detailed information concerning the group the reader is referred to the monograph of Thom and Church. The key below, taken from their work, covers only the more important species (or groups of species).

KEY TO THE GROUPS OF ASPERGILLI (THOM AND CHURCH)

A. Conidial heads green or yellow green.
  1. Stalks smooth.
    a. Vesicle cylindrical clavate, stalks coarse. *A. clavatus.*
    b. Vesicle flask-shaped or globose, not cylindrical clavate.
      aa. Sterigmata in one series.
        I. Conidia mostly elliptical and more than 4μ in long axis. Perithecia commonly found, yellow in color. *A. glaucus* group.
        II. Conidia mostly globose, 4μ or less in long axis; conidial chains in narrow, solid columns. *A. fumigatus* group.
      bb. Sterigmata in two series.
        I. Conidial chains in columns. *A. nidulans.*
        II. Conidial chains in radiate heads.
          a′. Heads blue green. *A. sydowi.*
          b′. Heads glaucus, green, or yellow-green to buff. *A. versicolor.*
  2. Stalks pitted (often rough as seen with low magnifications). *A. flavus-oryzae* group.

- B. Conidial heads never green.
  - 1. Stalks smooth.
    - a. Conidial heads avellaneous or in brown shades.
      - aa. Conidial heads in columns.
        - I. Stalks colorless or nearly so. *A. terreus.*
        - II. Stalks yellowed (especially in outer layer). *A. flavipes* group.
      - bb. Conidial heads radiate, stalks colored. *A. ustus.*
    - b. Conidial heads white (or slightly yellowed in age).
      - aa. Stalks yellowed, conidial chains in columns. *A. flavipes* group.
      - bb. Stalks colorless; heads mostly radiate or globose. *A. candidus* group.
    - c. Conidial heads brown to black. *A. niger* group.
    - d. Conidial heads orange to umber. *A. wentii.*
  - 2. Stalks pitted (or apparently rough).
    - a. Heads yellow to ochre, radiate.
      - aa. Heads in bright yellow colors. *A. sulphureus.*
      - bb. Heads in ochraceous shades. *A. ochraceus* group.
    - b. Heads orange to umber; conidia rough with tubercles and bars of color. *A. tamarii* and allies.

Aspergillus species are found growing on a wide variety of substrates. They are quite numerous in soil, and particularly so on dried vegetable matter, as hay and grains. They can apparently tolerate very high osmotic pressures and get along with surprisingly small amounts of moisture. In contrast with Penicillium species, they have as a group a rather high optimum temperature, and in a general way we find the former group growing in cool places on the same sort of materials that the latter will grow on at a higher temperature. Penicillium predominates in northern soils, Aspergillus in the tropics. Most Aspergillus species can grow at body temperature and some species are pathogenic, particularly for birds which have a high body temperature. It is important to remember this sensitivity to temperature when incubating cultures of these molds.

A. glaucus.—The term *Aspergillus glaucus* has been applied rather loosely to various different green species of Aspergillus. Thom and Church make of it a group of species, and abandon the specific name "glaucus." These species they differentiate mainly on the basis of the characters of the ascospores. But the name is commonly used to designate species of Aspergillus with bluish-green spores, readily producing yellow perithecia which are formed on the surface of the colony, suspended by the aerial mycelium. This group is found on a wide variety of substrates, and is one most frequently encountered as a contaminant in bacteriological work.

The general appearance of the organism is shown in Fig. 44. At first only conidia are formed, and the colony is green in color. But after a few days the yellow perithecia appear, and in old cultures the whole surface may be yellow in color rather than green. A section through one of the perithecia is shown in Fig. 45. Asci in various stages of development are scattered through a pseudoparenchyma. The appearance of the asci can best be determined by crushing perithecia in a drop of water under a coverglass. They appear as clusters of cells tightly held together by the membrane of the ascus (Fig. 46, *a*). The mature ascospores are elliptical in form and show a very characteristic double ridge engirdling the cell (Fig. 46, *b*). The vesicles vary considerably in form from slight expansions of the upper end of the conidiophore to more nearly globular forms (Fig. 46, *c*). The arrangement of the sterigmata is also somewhat variable, sometimes radiating, sometimes closely packed together on the upper surface of the vesicle. The conidia tend toward an oval form.

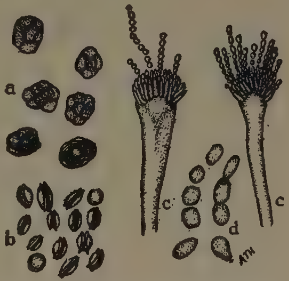

FIG. 46.—*Aspergillus glaucus.* *a.* asci expressed from a perithecium. *b.* free ascospores. *c.* sporeheads, showing arrangement of sterigmata. *d.* conidia.

A. fumigatus.—*Aspergillus glaucus* may be easily confused with *Aspergillus fumigatus*, since both species form blue-green conidia of much the same color. There is some question in the literature concerning the production of perithecia by *A. fumigatus.* Thom and Church conclude that typical members of this species do not possess a perfect form, but they include in the *fumigatus* group several closely related species which do. But certainly the pathogenic strains isolated from animals very rarely, if ever, produce perithecia. Thus, the absence of perithecia helps somewhat in differentiating *A. glaucus* from *A. fumigatus.* In *A. fumigatus* the vesicle is somewhat larger and typically club-shaped (Fig. 47, *b*), never globose as in *A. glaucus*. The conidia are much smaller and tend more toward a spherical form rather than oval; they are never prickly, as is true of some strains in the *A. glaucus* group. The conidia of *A. fumigatus* tend to change color from blue-green to a lavender-gray color rather rapidly with aging of the colony, which is not pronounced with *A. glaucus*. Finally, the higher optimum temperature of *A. fumigatus* may be mentioned as a differential character.

Aspergillosis.—*Aspergillus fumigatus* is an important pathogen, especially for birds. Probably our earliest definite records of mycotic infection in animals concern aspergillosis of the air sacs in birds. The disease is common enough in domesticated birds, pigeons, chickens, and ducks, to be of some economic importance. Under the name "brooder pneumonia" it sometimes occurs in epidemic form in little chicks. Autopsies of wild birds found dead have revealed many cases in all orders of birds, not only seed eaters, but also insect-eating birds and aquatic species. Fox,[18] in autopsies at the Philadelphia Zoological Garden, found cases in practically all of the groups of birds, the disease being responsible for death in from 0.6 per cent of the cases (pigeons) to 40 per cent of the cases (penguins). The fungus is also known to invade birds' eggs during incubation and to infect the embryos.

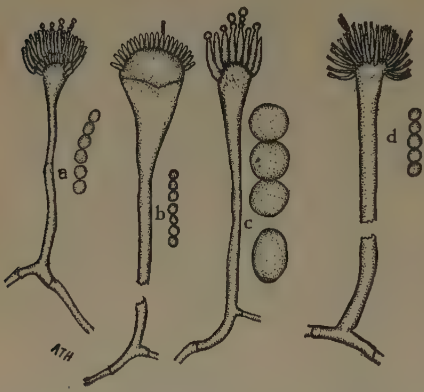

FIG. 47.—Conidiophores and conidia of species of Aspergillus. *a. Aspergillus nidulans. b. Aspergillus fumigatus. c. Aspergillus oryzae. d. Aspergillus wentii.*

Three types of infection occur in birds: infection of the air sacs, a pneumonic form in the lungs, and a nodular form in the lungs. The first type is a superficial infection of the epithelium lining the air sacs, which may pass into the wings or into the abdominal cavity. The wall of the sac becomes much thickened, a thick mat of mycelium covers its surface, and spores usually develop, so that the inner surface of the sac wall has a green color. In the pneumonic form a diffuse infiltrative lesion develops, the lung tissue is consolidated, and has a grayish white color. In the nodular form, isolated masses of infiltrated tissue occur, with necrosis in the center, much resembling advanced tubercles in their gross appearance. In those lesions developing in internal tissues not freely exposed to the air, no spores may be found.

The disease in domestic birds, especially when it occurs in epidemic form, can usually be traced to feeding moldy grain. It may be due to damp quarters in which straw or other material has become moldy. Infection of the eggs is usually due to moldy nesting material. Experiments have shown that if the eggs are carefully cleaned of their fatty coating, infection will not take place. The infected eggs may be detected by candling.

Primary lesions of the lungs also occur in various domesticated mammals, though not so frequently as in birds. Cattle, sheep, and especially horses are known to develop aspergillosis. As with the birds, infection comes from contaminated hay or grain, the spores being inhaled. In some cases particles of inhaled vegetable matter have been found in the lesions. The pulmonary lesions may be either nodular or pneumonic as in birds.

Aspergillus fumigatus is also pathogenic to man. The majority of cases described are infections of the external ear.[40] The earwax, like the fat on the surface of eggs, seems to favor germination of the spores. The extent of the disease may vary from a mere plugging of the ear canal with mycelium, leading to impaired hearing, to ulceration and suppuration of the walls of the canal, or even to penetration of the drum and invasion of the middle ear. The milder cases are the more numerous. Other species of Aspergillus may also produce this condition, particularly *A. nidulans* and *A. flavus*. According to Siebenmann [40] in Germany about 1 per cent of all ear cases are Aspergillus infections, but they cannot be nearly so frequent in this country. Aspergillosis of the ear is said to be particularly frequent in India.

Infection of the lung also occurs in man, but is quite rare. The majority of cases have been reported in France, though the disease is also well known in Germany. The disease may be primary, or secondary to some other condition, particularly tuberculosis. Lang and Grubauer [21] have reported a case in which bronchiectasis was apparently a predisposing cause. Secondary cases are more common than primary ones. It is quite possible that some of the cases reported as primary may have really been secondary to some other disease whose traces were obliterated by the aspergillosis. On the other hand, it is quite probable that some cases of primary pulmonary aspergillosis are overlooked, being mistakenly diagnosed as tuberculosis. Clinically the disease resembles tuberculosis very closely, perhaps advancing somewhat more rapidly. According to Lapham,[22] cases of primary aspergillosis give positive tuberculin reactions, while conversely Nicaud[30] found an advanced case of tuberculosis gave positive cutaneous reactions with an extract of *A. fumigatus.* Extensive cavity formation occurs in the lungs. The diagnosis is made by finding the mycelium in the sputum, and by isolating the organism in pure culture, which can readily be done by the use of dextrose-tartaric agar. The prognosis is not good. Internal administration of iodides is said to be of value, even curative, in some cases.

A considerable number of cases of primary pulmonary aspergillosis were reported in France some years ago, especially by Renon.[36] In these cases the infection was an occupational disease occurring in individuals engaged at that time in occupations which peculiarly subjected them to the possibility of inhaling large numbers of spores. These were the "gaveurs des pigeons" who fatten squabs for the market by filling their mouths with grain, chewing it fine, and then with their tongues forcing the mass into the esophagus of the birds; and the "peigneurs des cheveux" who prepare hair for the manufacture of wigs by mixing it with cornmeal to remove oil and then comb it out. In both cases the source of the infection is obvious enough, though in the first it is possible that the infection may have come from the bird rather than the grain, for some of the pigeons were found to have aspergillus infections of the mouth.

Experimentally inoculated into laboratory animals, *Aspergillus fumigatus* produces lesions which vary according to the virulence of the strain and the dosage.[39] Many strains isolated from air or vegetable matter show no pathogenicity. Strains freshly isolated from spontaneous infections may exhibit a surprising degree of virulence, a small dose of spores suspended in salt solution killing a pigeon overnight when inoculated intravenously. No lesions are apparent in such acute infections. With smaller doses or less virulent strains, multiple miliary abscesses occur in various viscera, especially the lungs. Intravenous inoculation into rabbits usually causes death within three to five days. Multiple minute abscesses in the cortex of the kidneys are the most striking lesions in these rabbits. Subcutaneous or intraperitoneal inoculations produce localized lesions which may not be fatal.

Experimental infections may also be produced by causing the spores to be inhaled. If one dusts spores into a tumbler and holds this over a pigeon's head for a minute or two, there develops a rapidly fatal haemorrhagic pneumonia. The author has not succeeded in producing any but very acute infections in this way. On the other hand, by feeding wheat which had been overgrown with the mold, he succeeded in two out of four pigeons in obtaining an infection of the air sacs very similar to the natural disease, death occurring in about six weeks.

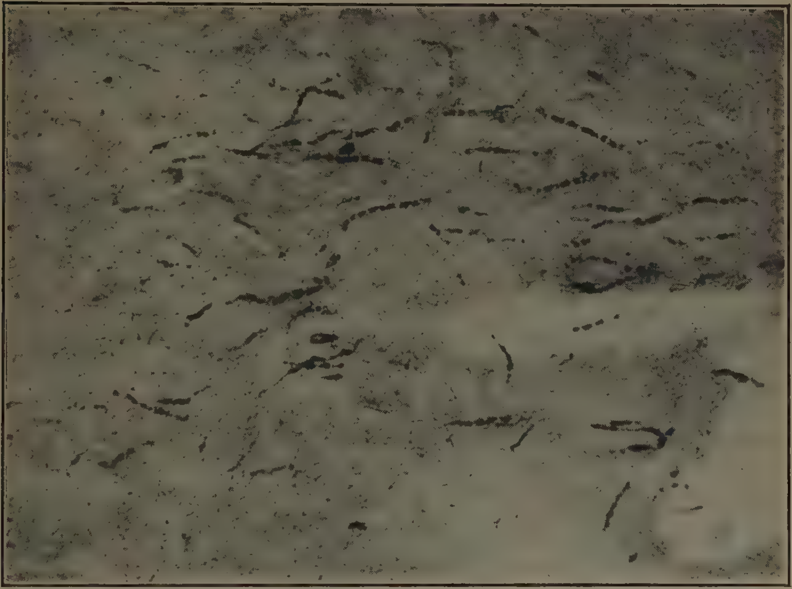

Fig. 48.—Section through the lung of a grouse (*Bonasa umbellus*) dead of spontaneous aspergillosis, showing filaments of mycelium. Gram-Weigert stain.

Microscopically both the natural and experimental lesions may vary considerably according to the virulence of the strain. Usually there is extensive necrosis in the vicinity of the organisms, with some suppuration. In the nodular lesions of the lungs there may be some production of fibrous tissue. In the lesions branched segmented filaments of mycelium may be found (Fig. 48), with conidiophores in various degrees of development where the fungus reaches a surface exposed to the air. In the miliary abscesses produced by intravenous inoculation one finds small masses composed of radiating filaments somewhat resembling a granule of *Actinomyces bovis*, save that the filaments are fewer and coarser.

Flavus-oryzae Group.—*Aspergillus flavus* and *Aspergillus oryzae* are yellowish-green molds. The former is recognized by its spiny, septate conidiophores. The conidia are smooth. *A. flavus* is a very widely distributed form, common upon grains and in soil. It is occasionally found in otomycoses, and strains isolated from that source may prove highly pathogenic for rabbits (personal observation).

Aspergillus oryzae is said to possess smooth non-septate conidiophores, though Thom and Church make no such differentiation, separating the species on the basis of color, *A. oryzae* being mainly yellow while *A. flavus* is more green, and on the more frequent occurrence of branched sterigmata in *A. flavus*. It would appear that a number of closely related varieties have been described under the name *Aspergillus oryzae*.

Aspergillus oryzae is one of the most important industrial ferments, having been used in Japan for many years in the "malting" of rice for the preparation of rice-wine, or saké, in the preparation of a fermented food, "miso," from soy beans, and in the preparation and manufacture of the dark brown "soya" sauce which has become familiar to us in Chinese restaurants.[54] It is also used as a source of a starch-splitting enzyme, "Takadiastase," produced commercially in this country. It produces both diastatic and proteolytic enzymes.

The mold is sold in Japan, generally grown in rice meal, under the name "koji." This commercial preparation is a rather mixed culture from which a number of organisms may be obtained. Several types may be obtained for different purposes, as "saké-koji" for wine making, "shoju-koji" for making soya sauce, etc. It is grown in the rice meal for some time, till numerous spores are formed, giving the product a yellowish-green color. During the growth the mass heats considerably. In saké production this "koji" is mixed with the rice mash to change the starch to sugar, and the alcoholic fermentation may be brought about by yeasts inoculated later. "Miso" is a sort of porridge or mush made by mixing mashed steamed soy beans, salt, and koji, and allowing it to undergo a brief fermentation. The bean is partially digested, the starch converted to sugar; some organic acids formed produce a characteristic flavor, while the high salt concentration prevents a growth of putrefactive organisms.

Soya sauce is made in a similar manner, but the fermentation is allowed to go further. The soy beans are half-cooked, mixed with wheat meal and "koji" and allowed to ferment three days, then mashed with the addition of salt. It is then allowed to ferment some time longer. The cell structure of the beans is broken down, the starch converted to sugar, and the proteins are broken down to substances which give the sauce its characteristic aroma and flavor. The salt content is very high (about 15 per cent), and though various yeasts and bacteria are present, they play only a minor role in the process. The final product bears a striking resemblance to meat extract both in composition and flavor.

Takadiastase is made in this country under patents taken out by Takamine.[43] The organism is grown in bran, which it heats, and as soon as the temperature begins to fall (indicating a maximal development of enzymes), the mixture is washed with water, the extracellular enzymes being thus extracted. From this extract the enzymes are concentrated by precipitation with alcohol. Though marketed mainly as a diastase, takadiastase really contains a number of enzymes.

A. nidulans.—*Aspergillus nidulans* is of a bright green color. The sterigmata are branched, and the conidia are given off in parallel rows, giving the spore head a rather characteristic form. Perithecia are formed which are pink to purplish in color. They are embedded in a cottony mass of mycelium. This organism is not infrequently encountered as a contamination in bacteriological work.

Aspergillus nidulans has not infrequently been encountered in cases of otomycosis. It has been found in one case of Madura foot by Nicolle and Pinoy,[31] and in a case of chronic suppuration of the skin back of the ear by Puestow.[35] In both of these cases the fungus was discharged in the pus as grains. Injections of large doses of spores intravenously into rabbits may give rise to infection.

Aspergilli as Causes of Splenomegaly.—Members of the *A. nidulans* group have recently been implicated as possible causes of certain types of splenomegaly. Pinoy and Nanta[33] interpreted certain deep-staining fibers which occur in nodules of fibrous tissue (Gandi-Gamma nodules) as filaments of mycelium, and succeeded in cultivating an Aspergillus closely related to *A. nidulans* from such spleens. These strains proved pathogenic for rabbits, producing kidney abscesses and pneumonia when injected intravenously;[34] Nanta[29] succeeded in producing splenomegaly by inoculating rabbits. The observations of Pinoy and Nanta have been confirmed by a number of others, but have been just as vigorously opposed. Hu, Reimann and Kurotchkin[20] seem to have clearly shown that the fibers in the Gandi-Gamma nodules are not mycelium, but iron-encrusted elastic tissue and collagen fibrils. Concerning the cultures obtained by various workers, three views have been expressed: that they are contaminations; that they are organisms accidentally present in the spleen, possibly found occasionally in the normal spleen; and that they bear an etiologic relationship to the disease.

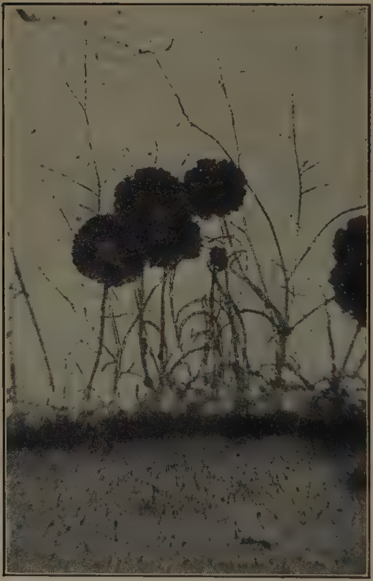

FIG. 49.—Spore heads of *Aspergillus niger*. Photomicrograph from a slide culture.

Aspergillus niger. — *Aspergillus niger* is quite the most common species. It is easily recognized by the rather high and widely separated conidiophores terminating in a relatively enormous black, globular spore-head (Fig. 49), easily distinguished by the naked eye. The conidiophore terminates in a large spherical vesicle, from which arise radially disposed primary sterigmata, each of which bears several secondary sterigmata. The conidia are black or dark brown, and spiny. The conidiophores arise from large and prominent foot cells. Details of the morphological characters are indicated in Fig. 50.

This species is of some importance as a cause of spoilage of foodstuffs. It grows by preference in substances rich in sugar and of an acid reaction, but may be found on a variety of substrates. It is said to cause a disease of the bark of cork oaks, and may frequently be found in cork stoppers, the use of which may give rise to decompositions of the bottled product. The author thus found *A. niger* the cause of spoilage of a laundry bluing composed of prussian blue, with oxalic acid used to stabilize the colloidal solutions. The mold oxidized the acid and caused the dye to be precipitated. The source of the infection was traced to the corks. Strains isolated from these bottles could tolerate enormous amounts of acid, growing in solutions containing 5 per cent oxalic acid.

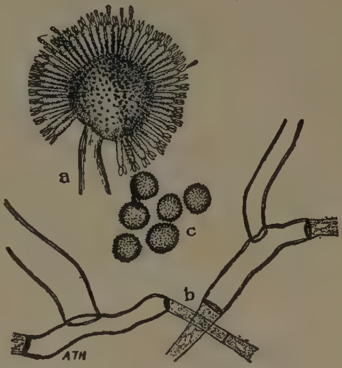

FIG. 50.—*Aspergillus niger*. *a.* vesicle and sterigmata. *b.* foot-cells. *c.* conidia.

Oxalic and citric fermentations.—Various molds produce both oxalic and citric acids from sugars, but strains of *Aspergillus niger* are apparently more active than other species, and have been used more extensively both for experimental and commercial purposes.

The formation of oxalic acid was rather extensively studied by Wehmer,[54] who believed that the acid was formed directly from sugar by an oxidation process:

$$C_6H_{12}O_6 + 9O = 3C_2H_2O_4 + 3H_2O.$$

Wehmer showed that the amount of acid formed could be greatly increased by adding calcium carbonate to the medium; and that in the absence of calcium carbonate, the acid formed gradually disappeared again, apparently being completely oxidized. Currie and Thom found that with some strains there was a lag between the curves for total acidity and for oxalic acid during the fermentation. This led to a search for another acid, which was identified by Currie[10] as citric acid. This was considered an intermediary product in the fermentation. According to Currie's concept, the oxidation of sugar by *A. niger* proceeds as follows:

$$\text{Carbohydrate} \longrightarrow \text{Citric acid} \longrightarrow \text{Oxalic acid} \longrightarrow CO_2.$$

Since citric acid is of some commercial value, attempts were made to use the fermentation on a commercial scale, and these have been successful, the acid being produced from sugar at a cost which permits competition with the product manufactured from citrus fruits. The details of the process are secret. The acid is formed from refined cane sugar.

The discovery of this reaction has led to a rather extensive investigation of the chemistry of the decomposition of sugar by *Aspergillus niger*, and the conditions which determine it. The most extensive of these investigations have been made by Bernhauer,[1] who showed that the yield of acid was influenced by numerous factors. The nature of the acid formed varies greatly with the strain; by careful selection strains may be obtained which yield almost entirely citric acid. The yield varies directly with the area of the mold mat, inversely with the depth of the liquid; aeration is an essential factor. He showed that the fermentation was not necessarily connected with the growth of the organisms, and that optimum conditions for growth were not optimum conditions for acid production; he therefore proposed to carry on the acid production in a two-phase operation, using one solution to produce the mat and replacing this with another for the fermentation.

The chemistry of the reaction was complicated by the discovery (by Molliard [27]) that in addition to citric and oxalic acids, gluconic acid is formed. This discovery has led to a wide variety of theories concerning the chemistry of the reaction, which have been amply reviewed by Bernhauer.[2]

Penicillium.—The genus Penicillium is characterized by the production of conidia from sterigmata much like those of Aspergillus, which are produced in clusters or whorls known as verticils, from short branches (metulae) given off, usually also in verticils, from the tip of the conidiophore. The appearance of a low-power view of the spore-head is somewhat like that of a brush; and the spore-head is called a *penicillus*, which is Latin for a brush.

Our knowledge of the Penicillia has been greatly extended in recent years by the extensive work of Biourge [4] and the still more exhaustive treatise of Thom.[46] The latter authority describes (including three closely related genera) over six hundred species! The differentiation of these is not nearly so simple as with the Aspergilli. In such a condition it is obvious that the precise determination of particular strains must remain a task for specialists. Fortunately, although widely distributed and a common source of contamination, only a few species are of any great practical importance.

In the earlier literature (and all too frequently even today) all green forms were referred to as *Penicillium glaucum.* This term has been used so indiscriminately for a variety of species that the name is as good as worthless. Although the color of the spores is of great value in diagnosis, more important are the size and structure of the conidiophores and conidia, and especially the character of branching. The appearance of the colony and biochemical reactions are also of importance. And it must be confessed that for one who is not a "penicillist," a knowledge of the natural substrate is a great aid in identification.

Thom recognizes three genera as being similar enough to Penicillium to be included in the group, containing species which have previously been described as Penicillia. These are Gliocladium, Paecilomyces, and Scopulariopsis.

Gliocladium.—Members of this genus form branched spore-heads resembling those of Penicillium, but the conidia become surrounded by a mass of slime binding together all of the spores of one spore-head into a rounded mass. But the conidia, in some species at least, are formed in chains, as in Penicillium.

Paecilomyces.—Paecilomyces is differentiated from Penicillium by its longer tubular sterigmata, the tubular processes being bent away from the axis of the sterigma, and by greater irregularity of the branching, which is only in part verticillate.

Scopulariopsis.—This group contains members which were formerly included with Penicillium, but which differ even more markedly than the above. They may form Penicillium-like branching systems, but frequently the branching is irregular, or the conidiophore may remain unbranched. The terminal branches which form the spores may not be constricted at their apex to a narrow tubular process, as is characteristic of Penicillium. And finally the large, thick-walled, spiny conidia with their ring at the base (Fig. 51) are quite different from those of Penicillium.

Scopulariopsis brevicaulis (formerly *Penicillium brevicaule*) is a common species of some importance. The spores are of a yellowish brown color. It is important as a cause of spoilage of various substances. Growing more slowly than other molds, it takes part in the final disintegration of the product. It is very active in proteolysis, producing ammonia abundantly in gelatin cultures. In the presence of sugars, it produces from arsenical compounds a substance, diaethylarsin, which has a very characteristic garlic-like odor. This reaction has been used as a test for arsenic, the reaction being said to be more delicate than the usual chemical tests. Only arsenious acid or its salts of the alkaline metals may be detected readily, salts of the heavy metals not so surely, and arsenic sulphide not at all.[54] But disagreeable odors may be produced on other substrates, described as ammoniacal, or like turnips or cabbage. According to Thom it is an important secondary invader and cause of spoilage of Camembert cheese and other dairy products. It may be found, along with other molds, in corks, and may give rise to very disagreeable odors in bottled products which have been stoppered with such contaminated corks, without any evidence of mold growth in the product itself. Various strains similar to *Sc. brevicaulis* have been isolated from cases of infection about the finger nails (onychomycosis); whether they are really pathogenic is doubtful.

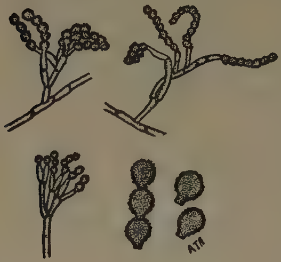

FIG. 51. — *Scopulariopsis brevicaulis.* Conidiophores and conidia.

The Penicillia proper are subdivided by Thom into four sections, and these are further subdivided into sub-sections. The basis for the primary subdivision is the nature of the branching of the spore-heads, whether this is symmetrical about the axis of the conidiophore, or asymmetrical. The symmetrical types are separated into three groups: the Monoverticillata, with a single whorl of sterigmata at the tip of the conidiophore; the Biverticillata-symmetrica, in which the verticils of sterigmata arise from short branches or metulae, which themselves form a verticil on the end of the conidiophore; and the Polyverticillata-symmetrica, in which three or more stages of branching occur. The asymmetrical forms make up the fourth group—the Asymmetrica. This classification can be more readily understood from a diagram than from descriptions (Fig. 52). These characters are subject to some variation, even in a single culture, but a dominant tendency will be manifest.

Monoverticillata.—Certain molds which formerly were classed as a separate genus, Citromyces, fall in this group. The name was given (by Wehmer) to two species producing citric acid from sugar. These forms were first considered as occupying a position transitional between Aspergillus and Penicillium, since some strains showed a slight swelling of the tip of the conidiophore. But Thom places them with the Penicillia, since numerous other typically penicillate forms have been found subsequently, and because the foot-cell characteristic of the Aspergilli is lacking.

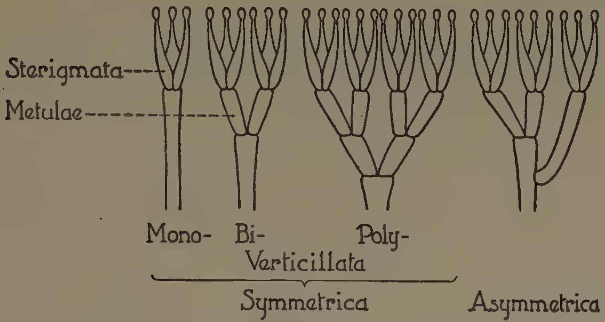

FIG. 52.—Diagram illustrating the different types of spore-heads of Penicillia.

Species of the Citromyces group were investigated at one time as a possible means of producing citric acid commercially, but without much success. As was indicated above, *Aspergillus niger* is now used more successfully for this purpose.

Biverticillata-symmetrica.—This section contains a fairly homogeneous group, of which *P. luteum* is the commonly known species. Ascospores, practically unknown in other Penicillia, are formed regularly by some strains of *P. luteum*, in loose masses of pseudoparenchyma, not in well-defined perithecia. This section is also characterized by the production of pigment, in the form of granules on the mycelium, which varies from yellow through orange to red, the color changing with the species, substrate, and age of the culture. *P. pinophilum*, producing stains on wood, belongs in this section, as does also the organism used in gluconic acid production (see p. 117).

Polyverticillata-symmetrica.—This is a small group of four species, none of which are of practical importance.

Asymmetrica.—The great majority of the Penicillia fall in the asymmetric group, including most of those of any economic importance. This section is divided into several subsections.

Velutina.—The colonies have a velvety appearance. *P. digitatum* and *P. expansum* are important species.

Brevi-compacta.—Colonies are partly velvety, partly woolly in texture, the penicillus biverticillate and showing characteristically a short compact base with divergent sterigmata and conidial chains. *P. stoloniferum,* growing on mushrooms and other fungi, and spreading by means of aerial runners, belongs here.

Lanata-typica, characterized by an abundance of aerial mycelium, giving the colony a woolly texture. Conidia appear first in the center of the colony after the felt of aerial mycelium has been established. *P. camemberti* is an important species.

Lanata-divaricata has the branches of the penicillus widely divergent, producing a looser type of spore-head than in the other groups. A series of species characteristically found in soil, of which *P. janthellinum* is typical, belong here. The soil species show yellow to red colors in the mycelium, the spores are green.

Funiculosa.—Trailing ropes or bundles of hyphae are found at the edge of the growing colony.

Fasciculata.—Species forming coremia, or tending to form coremia, are grouped together in this section. *P. expansum* and *P. italicum* are important.

Penicillia on Fruits.—The common soft rot of apples in storage is due to *Penicillium expansum.* If one stores such a rotten apple in a moist chamber, after some days the brown soft areas of the skin will become covered with numerous minute green spots of mold (Fig. 53). Examination of these with a lens shows that they are coremia, made up of bundles of conidiophores, and looking much like minute bunches of celery (Fig. 54). Coremia may also develop in cultures.

P. expansum is one of the most common species, and is probably the species that has been most frequently referred to as *P. glaucum.*

Although other organisms may at times cause a spoilage of apples, certainly in the great majority of cases this Penicillium is responsible. An injury to the skin is apparently necessary for infection to take place. The destruction of the apple is brought about by enzymes, which have been studied by Nobecourt.[32]

The spoilage of citrus fruits, particularly oranges, is caused by two species, *P. digitatum* and *P. italicum*. *P. digitatum* is sometimes referred to as *P. olivaceum*, since it forms conidia of an olive-green color. On oranges the olive-colored area is generally surrounded by a broad white zone of mycelium which has not formed conidia (Fig. 55). *P. italicum* produces spores of a light blue-green color. Both species may frequently be found growing on one orange. As with the apples, these organisms are wound parasites, and losses may be avoided by care in handling and packing. It is said that washing the fruit with a weak boric-acid borax solution may prevent infection.[45]

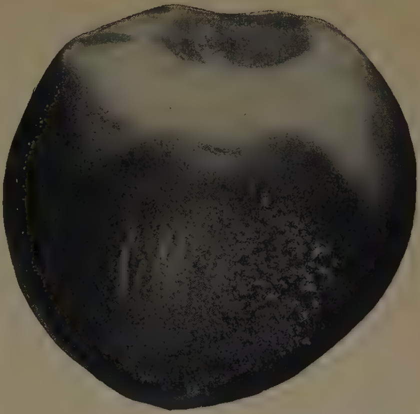

Fig. 53.—An apple affected with soft rot due to *Penicillium expansum*. The small white spots are coremia.

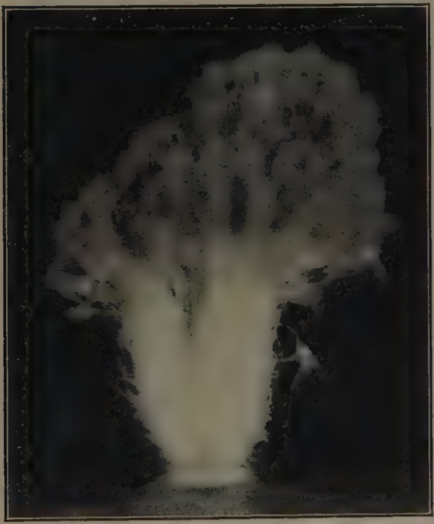

Fig. 54.—A coremium of *Pencillium expansum*. Photomicrograph.

Penicillia in Cheeses.—Two types of cheeses depend for their ripening and characteristic flavor on species of Penicillium.

Camembert cheese is a soft curd type of cheese, i.e., not so much water is allowed to drain from the curd, as in such cheeses as Cheddar or Roquefort. The curd is pressed into rather small cakes. This is necessary, as the ripening process depends very largely on a diffusion of enzymes from the surface to the interior of the cheese, which would require too long a time with larger cheeses. The mold is grown upon crackers or slices of bread, which are then dried and powdered. The crumbs are dusted upon the surface of the cheese, which is then stored in a damp atmosphere at a low temperature (50°–60° F.). The Penicillium grows over the surface, and after a time forms a gray-green coat of conidia. The ripening process depends upon proteolytic activities of the organism; when mature about 80 per cent of the nitrogenous matter has been made water-soluble.[5] The ripening begins on the surface and gradually proceeds toward the interior.

Other organisms, various bacteria, yeasts, and *Oidium lactis* are always present, and probably play some (though not an essential) part in the process. After the cheese has been properly ripened by the Penicillium, it may undergo a rapid spoilage by a secondary growth of these other organisms. Camembert cheese must therefore be marketed very shortly after it has ripened. They are usually therefore packed before they are fully ripened, the ripening process being allowed to continue in the container.

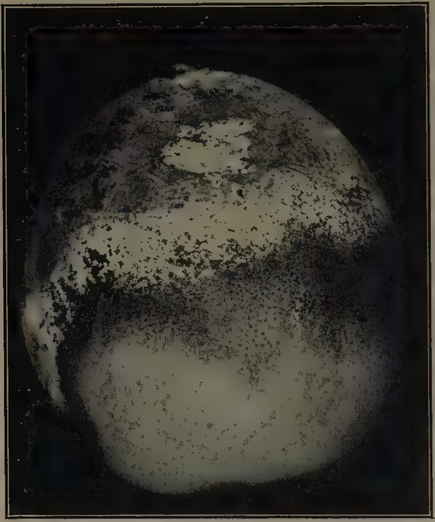

FIG. 55.— An orange overgrown with *Penicillium digitatum*.

Penicillium camemberti produces an abundant snow-white aerial mycelium, quite unlike the growth of most of the Fungi Imperfecti. Only after some days does a light gray-green color appear, indicating the development of conidia.

Roquefort cheese is a hard curd cheese, ripened by a mold whose mycelium grows throughout the curd substance. A preliminary lactic fermentation brought about by *Streptococcus lactis* is necessary to obtain sufficient drainage of the curd. The mold is grown as with *P. camemberti*, but the bread crumbs are kneaded into the curd. The curd is manipulated to leave spaces or cracks for the growth of the mold, or is stabbed in all directions after it is formed, in order to permit sufficient aeration for growth. But *P. roqueforti* can apparently get on with much less oxygen than is required by most molds, growing with as little as 5 per cent oxygen in the surrounding air.[48] The ripening is mainly due to fat hydrolysis, the characteristic flavor being due to the development of caproic acid.[9] A rather high salt content limits the growth of other undesirable organisms.[49]

Gluconic Acid Production.—The discovery that *Aspergillus niger* produces gluconic acid in addition to citric and oxalic acids, led to an investigation of this fermentation with other molds. May, Herrick, Thom and Church[23] have found that a species of Penicillium belonging to the *luteum-purpurogenum* group produced this acid to the exclusion of others. Since gluconic acid promises to be of some commercial value, May, Herrick, Moyer and Hellbach[24] have investigated the possibility of commercially using this fermentation. The yield is affected by the ratio of surface to volume of liquid, practically a ratio between 0.25 and 0.30 being best. Yields of 55–65 per cent were obtained. The mold was grown in aluminum pans placed in a sterilizable chamber.

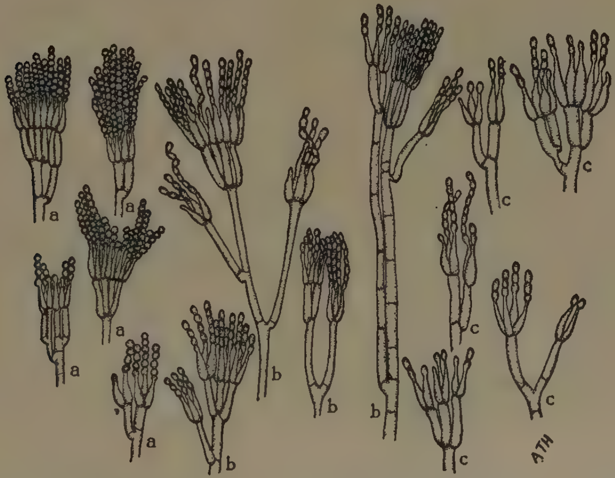

FIG. 56.—Penicillia from fruits. *a. Penicillium expansum.* *b. Penicillium italicum.* *c. Penicillium digitatum.*

Pathogenic Penicillia.—Although species of Penicillium have been found from time to time in various lesions, it is very doubtful that any of them are truly pathogenic.

Cephalothecium.—*Cephalothecium roseum* is one of the most common pink molds encountered. It may be readily identified by its clusters of three or four two-celled conidia formed at the ends of short conidiophores. It occurs widely upon a great variety of substrates, as fruits, wood, paper, and in soil, and is pathogenic for some plants.

Hormodendrum and Cladosporium.—One not infrequently finds as a contaminant on Petri plate cultures rather small dark olive-green colonies with a velvety surface. The reverse of the colony is almost black. These are most frequently *Cladosporium herbarum*, or *Hormodendrum cladosporoides.* The two are separated on the basis of uni- or bicellular conidia; but the bicellular conidia never develop until late, one-celled spores are formed first, and it is now generally considered that these are the same organism, which may or may not develop two-celled spores.

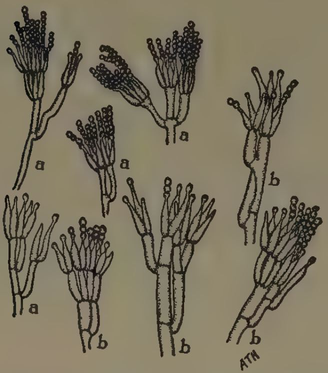

FIG. 57.—Penicillia from cheese. *a. Penicillium roqueforti. b. Penicillium camemberti.*

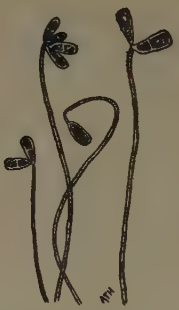

FIG. 58. — Conidiophores and conidia of *Cephalothecium roseum.*

This mold is the imperfect form of an Ascomycete, *Mycosphaerella tulasnei,* which is parasitic on various plants.

In Hormodendrum the conidia are formed in a peculiar manner. In molds like Penicillium and Aspergillus, conidia are formed by a constriction of the tip of the sterigma, which then forms a second spore in the same way, that pushes the first one ahead of it, and so on; thus the terminal spore in a chain is the oldest (and frequently the largest). In Hormodendrum, however, after the first spore has formed, the conidiophore fails to form any more; but the spore will bud out a second one, and then a third, and so on; thus the terminal spore in a chain is the youngest (and smallest); moreover, a spore may develop more than one new spore simultaneously, and thus we find the chains of conidia themselves branching (Fig. 59).

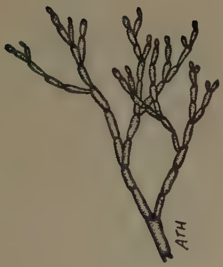

Fig. 59.—Spore-head of Hormodendrum.

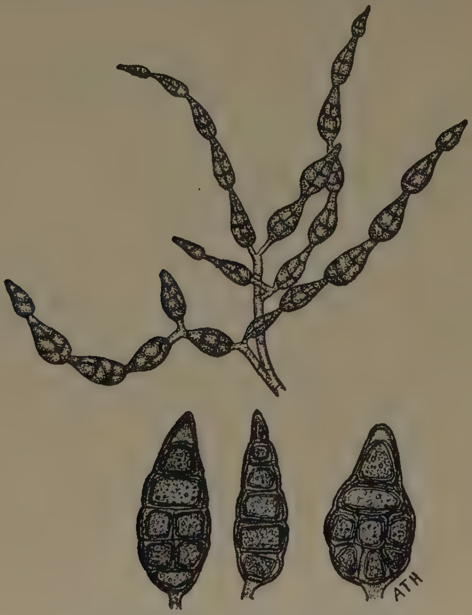

Fig. 60.—Conidia of Alternaria.

These molds are widespread. They are found especially frequently on decaying leaves and similar vegetable matter. They are of some importance as a cause of spoilage of malt and of stored tobacco. From time to time members of this group have been reported as causes of superficial skin lesions in man without any clear evidence of their pathogenicity.

There is some evidence that Hormodendrum or closely related forms may assume a yeast form known as *Torula nigra* or *Monilia nigra* (q. v., page 165).

Alternaria.—Members of this genus are also frequently encountered by the bacteriologist. They form dark olive-green or brown colonies similar to those of Hormodendrum, save that the aerial mycelium is much looser, forming a more woolly type of growth.

Molds of this group may be recognized by the peculiar spores of rather large size, multichambered, and composed of a number of cells. These occur in chains, sometimes with short stretches of mycelium between the spores. There are a number of species, many of which are plant pathogens. Stemphyllium and Macrosporium are closely related genera. For a discussion of the classification of this group, consult the article by Elliott.[16]

Species of Alternaria have been found growing in pus of superficial wounds, and it has been suggested that they have some pathogenic action. But this is probably not true, the organisms growing merely as saprophytes upon the pus, or more likely upon the cellulose of the dressings. Their relation to asthma has been mentioned on page 61.

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CHAPTER VI

THE DERMATOMYCOSES

THE term dermatomycosis may be used in a broad sense to denote any infection of the skin by fungi, but is generally used in a narrower sense to indicate a series of diseases caused by a group of fungi which practically never invade any other tissue. These fungi for the most part produce only a superficial infection, not tending to involve the deeper tissues or to spread to internal organs, but are nevertheless important because of their frequent occurrence and to a certain extent contagiousness. They may be divided into two main classes: First, those in which there is, strictly speaking, no true infection, but merely a saprophytic growth of fungi on the epidermis or hairs (Pytiriasis versicolor, Erythrasma, Piedra, and Pinta); and second, those in which there is an actual invasion of the tissues with an inflammatory reaction. The fungi concerned in the first class of disease consist of several unrelated species of Fungi Imperfecti; those which cause the second group comprise a series of closely related species with numerous intermediary forms which make up a fairly homogeneous group. This group is frequently referred to as the Dermatophytes.

A clear knowledge of the latter group of fungi can be obtained only with considerable difficulty. The subject is quite complicated for several reasons. Attempts have been made to classify the causative fungi according to the nature of the disease which they produce; but it is now quite clear that the same disease may be produced by several different species. On the other hand, attempts at a purely botanical classification based upon morphological characters has been somewhat unsatisfactory because of the pronounced pleomorphism of the organisms. The parasites exhibit a certain amount of geographic specialization, species isolated from cases in one part of the world being different from those obtained in other parts. Some of the parasites occur only on man, some only on certain lower animals, but a few may be transmitted from animals to man. A very large number of species have been described. For these various reasons an extensive knowledge of the group can only be obtained after long and intensive study. The subject is one for the specialist. We can do no more here than point out some of the more important general characters of the diseases and their etiologic agents, and describe a few of the more common species.

Although a very large number of investigators have contributed a voluminous literature to the subject, the work of one man, Sabouraud, overshadows all the rest and dominates the field. To his long-continued, patient, and intensive studies we are indebted for what order and comprehensiveness we have in our knowledge of the skin fungi. We shall follow, in the main, his classification, which, whatever it may lack in botanical correctness, has the advantage of being practical and in general use.

Position of the Dermatophytes.—Some of the dermatophytes occasionally form peculiar twisted masses of mycelium which have been looked upon as abortive attempts to form asci. (These are referred to by French authors as "accessory organs.") Matruchot and Dassonville [6] noted a similarity between certain of the dermatophytes and some species of the Gymnoascaceae, and claimed to have produced experimentally a ringworm with a species belonging to this family of the Ascomycetes, *Ctenomyces serratus*. The Gymnoascaceae are characterized by the formation of asci, not in a compact and well-defined perithecium, but in masses surrounded by a rather loose meshwork of protective mycelium, the peridium. More recently Nannizzi [8] has claimed that peridium-like organs are produced by the skin fungi when grown on feathers, the pelts of guinea pigs, and other substrates, although no true asci were formed. Tate [12] could not confirm this observation. On this rather slim basis several authorities have classified the dermatophytes with the Ascomycetes. It would seem safer and more logical, however, to group them with the Fungi Imperfecti. Because of the varied types of reproductive bodies formed, however, it is quite difficult to assign them a position in Saccardo's classification. Vuillemin groups them with his Arthrosporineae, Ota and Langeron [9] with the Conidiosporales of Vuillemin's classification.

Types of Spores.—In the hair and skin the dermotaphytes reproduce only by a fragmentation of the mycelium into its component cells. The so-called spores found in the lesions are therefore to be looked upon as oidia; in much of the French literature they are referred to as arthrospores.

But in artificial cultures a variety of other structures appear. Within the last few years these have been rather intensively investigated with a view toward establishing a purely botanical classification. Besides oidia, there may be found in cultures of various species, chlamydospores, aleuriospores, and spindle spores. In addition, certain peculiar types of articulation or of branching of the mycelium itself have received some attention. For detailed descriptions of these structures the reader is referred to the papers of Ota and Langeron,[9] and of Tate.[12]

Oidia, or arthrospores, are formed in cultures less regularly than in the skin, generally only at the tips of filaments of mycelium near the margin of the colony. They were referred to by Sabouraud as "endoconidia" to distinguish them from the spores formed by the aerial mycelium which he designated "ectoconidia." The latter are being more frequently known by the name introduced by Vuillemin, "aleuriospores." They are like conidia in their origin and appearance but differ in that they are not liberated when mature, being set free only when the mycelium that has formed them has disintegrated. They may be lateral or terminal, sessile or pedunculated, and may or may not be separated from the mycelium by a septum.

Chlamydospores are those arising in the vegetative mycelium. They are rather large, thick-walled cells, formed sometimes in the course of the mycelium (intercalary), sometimes laterally on short stalks. They may be irregular in form, many approaching a spindle-form, and sometimes are composed of several cells. Thus there are many transitions between these chlamydospores and the spindle-spores. The latter, known in French literature as "fuseaux," are formed by the aerial mycelium, abundantly in some species, rarely or not at all by others. They are generally composed of several chambers, each containing a cell. These various types of spores are illustrated in Fig. 61 from Tate's review.

One may find all transitions between the above structures. It is obvious that there will be many cases where it will be impossible to decide whether a given structure is a lateral chlamydospore or an aleuriospore, a terminal chlamydospore or a spindle. None of the structures are as definite or tangible as the conidial fructifications of the molds discussed in the preceding chapter.

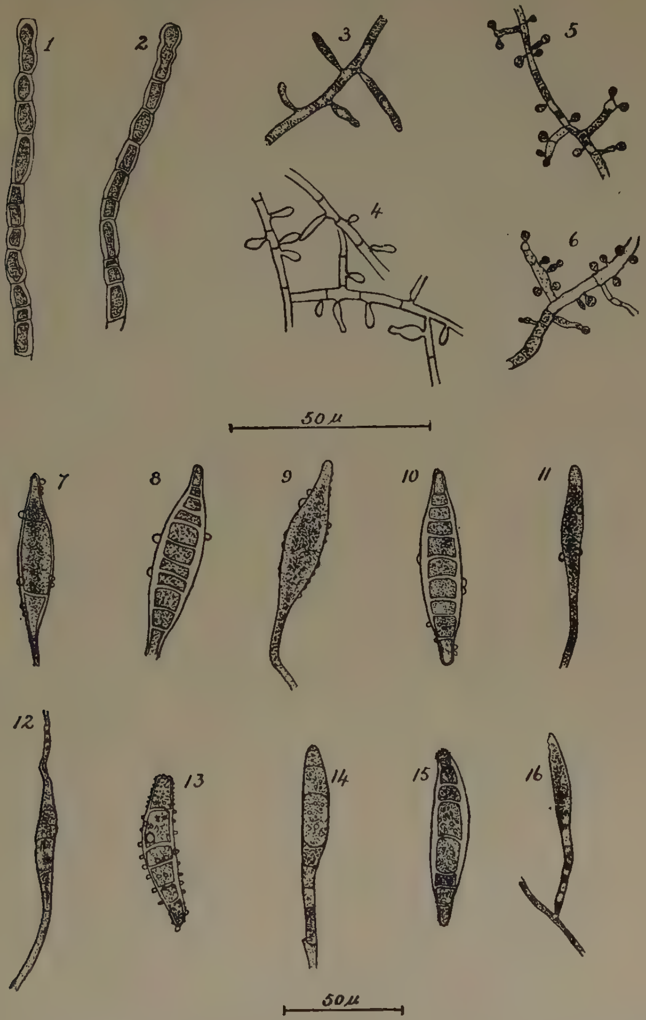

Fig. 61.—Types of spores formed by ringworm fungi. 1, 2, arthrospores; 3–6, aleuriospores; 7–16, spindle-spores. After Tate.

Cytology.—Grigoraki[2] has studied the cytology of the ringworm fungi. They differ from most of the other pathogenic fungi in containing several nuclei to each cell, generally from two to six. These divide by amitosis. Aleuriospores contain but a single nucleus, whereas chlamydospores, arthrospores, and the individual cells of the spindles are multinucleate. Mitochondria and metachromatic granules are also present.

Cultural characters.—The identification of the various species of dermatophytes is made very largely by the appearance of the colonies. Since this is subject to some variation with the composition of the medium, Sabouraud insists that cultures shall be made upon his "proof" agar. Others claim that this is not so necessary, ordinary commercial maltose and peptone serving equally well. Plaut and Grütz[10] offer several alternative formulas which they claim will serve in place of Sabouraud's medium.

The colonies may be smooth and waxy if no aerial mycelium is formed, or with a chalky surface, or of velvety or woolly texture, depending upon the abundance and height of the aerial mycelium. They may be yellow or rose or violet in color, but are usually white. They may be irregularly folded to form cerebriform masses, or regularly folded in radial form.

But these characters are not constant. Pigment production is usually lost rapidly on subcultivation. And the texture of the colony is frequently altered by the gradual or sudden appearance of an abundance of sterile aerial mycelium, at first as localized tufts on various parts of the colony, but rapidly overgrowing the whole colony. Old laboratory cultures are thus frequently quite atypical. From these considerations it will be seen that the precise determination of species is a matter requiring some experience and judgment.

Enzymes.—The biochemical activities of the skin fungi have only recently been investigated. Tate[13] found that all of the species which he investigated were proteolytic, and secreted lipase, urease, maltase, and diastase. None contained invertase, inulase, lactase, or zymase. The proteolytic enzyme resembled trypsin, acting in an alkaline medium; it did not digest coagulated egg. None of the strains studied could hydrolyze keratin. The fat-splitting activities of ringworm fungi have been studied by Mallinkrodt-Haupt,[5] who found that the species studied could grow in mineral solutions with neutral fats as the sole source of carbon, if these were of animal origin, but showed little or no growth with vegetable oils.

Classification.—In 1923 Ota and Langeron [9] proposed a purely botanical classification of the dermatophytes based upon the spores in cultures. This classification has received considerable attention, and has been followed to some extent in subsequent literature, especially French. Ota and Langeron criticized the classification of Sabouraud, in general use until that time, as being based largely upon clinical characters of the disease, and therefore as being unscientific. They note that fungi which may appear quite diverse in their appearances in cultures may give rise to the same sort of disease, and may appear the same in the skin or hair. They group their genera together in a subfamily, the Closterosporeae. The genera are subdivided according to the following key:

- Aleuriospores numerous, well developed, isolated or in clusters.
  - No spindle spores or accessory structures. *TRICHOPHYTON.*
  - Spindle spores and other accessory structures (areas of much branched and spirally twisted mycelium) present. *SABOURAUDITES.*
- Aleuriospores rare and imperfect, isolated, rarely in clusters.
  - Spindle spores numerous and well developed. *EPIDERMOPHYTON.*
  - Spindle spores absent.
    - Arthrospores, accompanied by accessory organs. *GRUBYELLA.*
    - Arthrospores, without accessory organs.
      - Arthrospores in chains. *BODINIA.*
      - Arthrospores not well defined. *ENDODERMOPHYTON.*

Another genus, Ateleothylax, was created to include a single species in which ascospores were claimed to have been found by Chalmers and Marshall.

As was pointed out in the preceding chapter, it is very doubtful whether the characters of the non-sexual spores of the Fungi Imperfecti really indicate any phylogenetic relationship. It is quite possible that even such well-defined groups as Aspergillus and Penicillium may be composed of species quite heterogeneous in origin; with such ill-defined reproductive bodies as those of the dermatophytes this becomes a probability. Any classification of the Fungi Imperfecti must be one of convenience rather than based upon systematic relationships. This being true, it seems to the author that the original classification of Sabouraud is much to be preferred.

Sabouraud recognized three main genera of dermatophytes, Microsporum, Trichophyton, and Achorion. The last was distinguished by the production of peculiar yellow crusts, or scutula, in the scalp. The other two were originally distinguished by the size of the spores in the hairs, but have since been differentiated on more distinct characters. The genus Trichophyton was further subdivided into subgenera upon the basis of the position of the fungus with regard to the hairs—Endothrix trichophytons, growing entirely within the hairs; Ectothrix trichophytons, growing mainly on the surface of the hairs; and later Ecto-endothrix, or Neo-endothrix trichophytons, showing mostly a position within the hairs, but in some hairs a growth on the surface occurs. To these genera must be added two others, which do not invade the hairs, Epidermophyton and Endodermophyton.

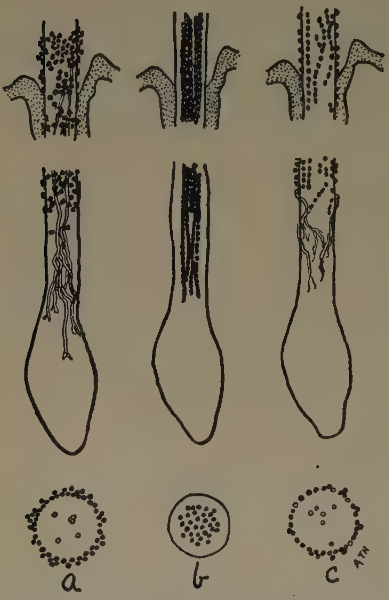

Fig. 62.—Diagram showing the relations of ringworm fungi to the hairs. *a*. Microsporum. *b*. Endothrix Trichophyton. *c*. Ectothrix Trichophyton.

The key to the main groups of dermatophytes presented below is based very largely upon clinical characters. The main groups write their own label, so to speak, upon the skin, and their identification is certainly easier if one has also seen the patient. In using Sabouraud's system of differentiation, it is obvious that the inspection of hairs is to be preferred where such are involved. For this purpose it is of course essential to select diseased hairs. Too frequently a bunch of perfectly normal hairs is sent to the laboratory for diagnosis! The organism is found in the lower part of the hair, that which is within the follicle and for a relatively short distance above the skin level. Hairs which have broken are of course more likely to show an extensive development of the organism than those which are not. Infections with *Trichophyton* *acuminatum* cause the hairs to break off flush with the skin, the stumps appearing as black spots in the follicles. These should be extracted with forceps for examination. In all cases the fungi first invade the hairs in the sheath. Whether eventually it will be found within the shaft or without would appear to depend largely upon the duration of the infection, which in turn is correlated with the degree of inflammatory reaction. In any case, it is well to remember that the differentiation according to position of the fungus in or on the hair is not an absolute one. In endothrix forms there will be some spores outside the hair, in ectothrix types some within.

KEY TO THE DERMATOPHYTES

Lesions of the Scalp

A. Yellow crusts or scutula present. Hairs tend to split longitudinally and not break off transversely. *ACHORION.*
  1. Colonies yellow, waxy, no aerial mycelium. Mycelium breaking up into oidia, and forming swollen tips to filaments, but forming no conidia or true spindle spores. *Achorion schoenleinii.*
  2. Colonies white, downy. Conidia and spindle spores formed. *Achorion quinckeanum.*

B. No scutula present. Hairs tend to break off square.
  1. Suppurative reactions, as follicular abscesses, pustules, or kerion, absent.
    a. Hairs broken off at a uniform height, several millimeters above the skin. Much scaling of the epidermis, highly contagious. Spores on outside of hairs, angular, forming a mosaic. In cultures, conidia without stalks. *MICROSPORUM.*
    b. Hairs mostly broken off flush with the skin, leaving black points. Little scaling of the epidermis. Not so contagious. In cultures conidia on short stalks.
      aa. Grows entirely within the hair, both mycelium and spores. *Endothrix TRICHOPHYTON.*
      bb. Grows mainly within the hair, but a few mycelial filaments and spores can be found on the exterior. *Neo-endothrix TRICHOPHYTON.*
  2. Suppurative reactions occur. Lesions of the smooth skin also frequently present. Of animal origin. Fungus grows in and on the hair, spores mostly external, arranged in rows, not in a mosaic. *Ectothrix TRICHOPHYTON.*
    a. Spores 5–8μ in diameter. Section *Megaspores.*
    b. Spores 3–4μ in diameter. Section *Microides.*

Lesions of the Smooth Skin

A. Eczema-like lesions confined to moist parts, as inner surfaces of thighs, axillary region, between fingers or toes, soles of feet. Parasite not found within hairs. *EPIDERMOPHYTON.*

B. Lesions not as above, generally involving backs of hands and arms, or face and neck.

1. Lesions form intricate patterns of concentric rings with marked scaling. Parasite not found in hairs. Disease restricted to Far East and Pacific Islands. *ENDODERMOPHYTON.*
2. Lesions are reddish patches, not raised above the skin level, round to irregular in form, darker at the border, forming rings. Tending to heal in the center, new attacks may occur, forming concentric rings.
   Generally *MICROSPORUM.*
   Sometimes *Endothrix TRICHOPHYTON.*
3. Lesions are elevated plaques, reddish, round or oval, scaly. Pustules frequently present at the border. *Ectothrix TRICHOPHYTON.*

Favus.—Favus is caused by various species of Achorion, particularly *Achorion schoenleinii.* The disease occurs not only in man, but also in various lower animals, particularly mice, but also cats, dogs, chickens, and some others. The great majority of human cases are contracted by direct or indirect contact with preceding cases, and are due to *A. schoenleinii*, but some of the species infecting lower animals will also produce the disease in man.

The disease occurs particularly in the poor and the unclean. It has almost disappeared in the more advanced countries, but is prevalent in southeastern Europe and has increased in eastern Europe since the war. It occurs much more frequently in children than in adults.

The lesions occur most frequently on the scalp, though other parts of the body surface may be affected, but in the latter case the disease has usually been carried from the scalp. The fungus grows between the outer cornified layer of the skin and the inner layer of epithelial cells (Malpighian layer) and forms a very characteristic yellow cup-shaped mass, the scutulum. The infection begins in a hair follicle, and the hair is usually seen projecting from the scutulum. When the latter is pulled off, there is left a raw, sometimes suppurating surface. By growth and coalescence of the scutula, there may be formed an extensive crusted layer on the scalp. The hairs become opaque and dull, and finally drop out, leaving bald spots.

A section through the scutulum shows a radiating feltwork of mycelium, which tends to die off in the center, leaving a granular debris, and to form spores at the periphery. The fungus also invades the shaft of the hair, forming parallel bundles of mycelium through its center.

The organism may be demonstrated by a microscopic examination of either the scutulum or a hair. A small portion of the former should be crushed under a coverslip in a strong sodium hydroxide solution, the latter should also be immersed in strong alkali. In the lesions the mycelium is very irregular, its component cells varying considerably in size and form. The cells tend to break apart, giving the filaments an articulated appearance, and the terminal portions of the filaments give rise to a series of round oidia. In a preparation from a scutulum as above described, these elements become mixed together and one finds a melange of rounded cells, short oval cells, and longer and shorter fragments of mycelium. The fungus has a similar structure in the hair; one finds long, articulated filaments of cells of varying size, the terminal portions ending in a series of rounded "arthrospores." A very characteristic feature is the degeneration and disappearance of the fungus in the hair, leaving a series of air bubbles.

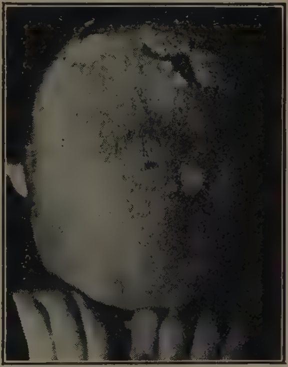

Fig. 63.—Favus of the scalp, showing crusts or scutula.

Cultures are obtained by inoculating minute portions of scutula or hairs on the surface of agar plates. As these cultures are likely to be contaminated with bacteria, a large number should be prepared to increase the chance of obtaining some colonies reasonably pure. The particles of material are transferred to the surface of the agar with a needle, making three or four widely separated inoculations on a plate. Peptone agar without sugar is said to be best for the first cultures, though after a time the organism will grow better with sugar. The optimum temperature is 30° C. The various strains vary considerably in rapidity of growth.

The colonies on agar are at first yellowish and waxy in appearance. As they grow older they become much wrinkled and develop a whitish aerial mycelium (some strains may fail to do this). In cultures the organism is extremely pleomorphic. In slow-growing strains, the mycelium may become articulated and numerous chains of arthrospores appear as in the lesions. In more rapidly growing strains there occurs an extensive development of mycelium which is, however, very irregular, forming in some places thick, irregular masses having little resemblance to ordinary mycelium. These are called the ameboid forms. Large yellowish thick-walled cells may appear in the course of the mycelium. Finally, filaments at the periphery of the colony end characteristically in a branched cluster of swollen cells. The latter structure is referred to as the favic "chandelier."

The organism is apparently of variable pathogenicity for lower animals. Typical scutula have been produced by inoculation of the skin in mice. Intravenous and intraperitoneal injections into rabbits produce nodular lesions of the lungs or peritoneum respectively.

Cases of favus contracted from lower animals are not nearly so frequent as the purely human form. Of these the mouse type is more frequently seen. It occurs on the smooth skin, rather than the scalp. Multiple scutula may develop, but the disease does not tend to spread and progress as in the infections with *Achorion schoenleinii.* The parasite of mouse favus is called *Achorion quinckeanum,* but has little in common with *A. schoenleinii* save its ability to form scutula on the skin. According to Plaut, it belongs rather in the genus Microsporum.

Ringworm.—Ringworm is a parasitic affection of the skin due to various fungi belonging to the Trichophytons or Microsporum, occurring endemically in some centers of population and at times producing small epidemics. The disease is transmissible from man to man, from lower animals to man, and rarely from man to animals. It was formerly a very common disease, especially in children of the poorer classes. For a time in Paris special schools were maintained for children with ringworm. Partly as a result of a rapid means of treatment by Roentgen rays, the disease has greatly decreased in prevalence within recent years.

The disease is transmitted by means of the spores formed in or on the skin and hairs, either by direct contact or indirectly through combs, brushes, towels, etc. It may be contracted from animals also by direct contact, more frequently from the walls of stalls, litter about barns, etc. There is no clear evidence that the parasite is capable of living saprophytically upon vegetable matter, as is true of many of the other pathogenic fungi. But the spores can probably remain viable for long periods outside the animal body.

The disease may attack all parts of the skin surface. Clinically a division is made between ringworm of the hairy parts and ringworm of the smooth skin; ringworm of the nails also occurs. The nature of the infection varies considerably according to the degree of inflammatory reaction of the invaded tissues. Thus a wide variety of clinical types may be recognized. But the differences are more apparent than real; fundamentally the pathologic changes are the same in all of the types.

FIG. 64.—Ringworm of the smooth skin.

The infection begins in a hair follicle, from which focus it extends to the surrounding skin and into the hair. In the skin, in addition to redness due to congestion, there occurs scaling caused by an overproduction of epithelium in response to the irritation caused by the presence of the fungus, and an exudation of fluid and an accumulation of leucocytes. A very characteristic feature is the formation of concentric rings of inflammatory reaction. This is, of course, more apparent on the smooth skin. The formation of these rings has not been satisfactorily explained, but may be due to the same mechanism which leads to the formation of concentric rings of growth so frequently when molds are grown in Petri plate cultures. The infection tends to spread at the periphery and heal in the center. It is the formation of these advancing rings of inflammatory reaction which has given origin to the popular name "ringworm" (Latin, Tinea; French, teigne).

Microsporum Ringworm.—In Microsporum, although the mycelium invades and is found inside the hairs, the spores are formed on the exterior. There is some difference of opinion as to whether these spores are to be considered arthrospores (oidia), i.e., produced by fragmentation of the mycelium, or whether they are rather conidia. But they occur in irregular clusters, not in chains, and are closely packed together on the surface, forming a sort of mosaic. The individual cells are polyhedral in form, not round or cylindrical. In the Trichophytons, the mycelium may be within or without the hair, and the spores are formed in both places, but they are definitely produced by a fragmentation of the mycelium, and as a result appear in regular parallel rows or chains.

A further differentiation is seen in the mode of formation of the aleuriospores in artificial cultures, though this is not so reliable, since in both types pleomorphism is common and formation may be quite irregular. In Microsporum they are typically sessile, resting directly on the filament of aerial mycelium without any stalk. This is referred to as the acladial type, since it resembles fungi of the genus Acladium. In Trichophyton the spores are constricted at their base, so that there is apparently a short stalk connecting them to the mycelium. This is called the botrytis form of fructification, being reminiscent of molds belonging to the genus Botrytis.

Ringworm due to members of the genus Microsporum is frequently referred to as Microsporosis, to distinguish it from the ringworms caused by Trichophytons. Microsporosis is in the majority of cases a ringworm of the scalp, called "tinea capitis" by the dermatologists. It occurs most frequently in children; in stubborn and untreated cases it may persist for some years, but usually disappears at puberty. It is by far the most contagious of the ringworms. It is most prevalent in England and France, being the cause of 90 per cent of the cases of tinea capitis in the former country, and of 60 per cent of all the ringworms in Paris. It is less frequent in other countries.

The lesion consists of a reddened scaling area which tends to spread in the characteristic ring form. A number of such patches may form and coalesce, giving irregular areas. There is marked scaling of the epidermis. The hairs tend to break off transversely at a height of 2–6 millimeters, leaving the stumps somewhat thickened, whitish and opaque. Thus there appear a number of very characteristic irregular ("moth-eaten") bald patches covered by the short stumps of the diseased hairs, which are fairly uniform in height. Generally inflammatory reactions are not pronounced.

The diagnosis may be established by examining an epilated hair in a drop of sodium hydroxide solution. There will be found on the outside irregular clusters of the polyhedral cells ("ectospores") and in the interior of the hair short articulated filaments of mycelium tending to break up into short chains of "arthrospores."

The spores germinate readily on Sabouraud medium and give rise to a rather fine septate mycelium. After a few days the mycelium becomes distended here and there, the swollen cells developing into chlamydospores. These are very characteristic of the mycelium of Microsporum. The aerial mycelium is frequently peculiarly twisted, and gives rise to numerous lateral branches of short length and finally the acladial type of spores described above. Later long, narrow branches of the aerial mycelium become swollen at the ends and develop the pluri-septate spindle-spores. The latter may frequently form fine hair-like processes at their extremities, which when present, further serve to differentiate these fungi from the Trichophytons. Disarticulation of the mycelium into oidia occurs only in old cultures.

A number of species of Microsporum have been described. Castellani recognizes sixteen, of which four are of human origin, the remainder of animal origin. The great majority of cases are due to *Microsporum audouini* (132 out of 161 cases observed by Sabouraud), which is a strictly human variety. Of the cases of animal origin, those caused by *Microsporum caninum* (*M. lanosum*) are the most frequent. These two species may be differentiated by the characters tabulated below (from Plaut and Grütz).

Microsporum audouini	**Microsporum caninum**
Highly contagious, causing school epidemics.	Less contagious, may cause family epidemics.
Of long duration, resistant to treatment.	Of shorter duration, about 1 year.
Only the head is involved, exceptionally areas in the immediate vicinity.	Frequently also skin lesions at a distance from the head.
Inflammatory reactions mostly lacking if untreated.	Inflammatory reaction present without any irritation from treatment.
Cultures grow slowly.	Cultures grow more rapidly and colonies attain a larger size.
Colonies remain snow white.	Colonies become reddish in center when spindle spores are formed.
Only rarely transmissible to animals from cultures.	Rabbits and guinea pigs easily infected by cultures.

Further differentiation may be made on the basis of colony structure. In *M. audouini* there is a small central knob elevated above the surface of the colony. The latter is raised into radial furrows separated by four to six deep clefts. And the whole surface has a velvety texture. In *M. caninum* there is a central flat elevated portion surrounded by an elevated ring of aerial mycelium, and no folding. The whole surface has a more woolly texture.

Endothrix Trichophytons.—The pure endothrix Trichophytons are the strictly human types. Their infections occur mainly in the scalp, the majority of the cases of ringworm of the scalp not due to Microsporum being caused by members of this group of molds. They produce persistent infections without much inflammatory reaction. Although most cases occur in younger life, the infection does not, like microsporosis, tend to disappear at puberty, but may persist into adult life. Trichophytosis of the scalp is differentiated from microsporosis by the fact that the organism is found almost exclusively within the hair, the spores being arranged in chains; the hairs tend to break off at the skin level rather than a few millimeters above, leaving a smooth bald spot with few hair stumps and little or no scaling of the skin. In addition the disease almost always involves some part of the smooth skin as well. The disease is sometimes referred to by its French name, peladoide.

There are a number of species of endothrix Trichophytons. Castellani[1] recognizes fourteen. According to Sabouraud's statistics, 72 per cent of the cases due to members of this group are caused by two species, *Trichophyton acuminatum* and *Trichophyton crateriforme.* A third species, *Tr. violaceum,* is found more frequently in other countries, including America.

Trichophyton acuminatum produces large irregular bald patches, the hairs breaking off flush with the skin early after infection. The skin of the scalp in these bald patches may appear quite healthy. Some hair stumps may be present. These are dark in color, thicker than the normal hairs, and pull out with some difficulty. Microscopic examination reveals the chains of "spores" in the interior of the hair. They are round, oval, or cylindrical in form and are produced by simple fragmentation of the mycelium into its component cells. They are much larger than the spores of Microsporum, and have thick walls. It is noteworthy that the spores of this species, as well as those of *Tr. violaceum,* unlike the spores of most other pathogenic fungi, may be disintegrated by strong sodium hydroxide solution.

In addition to the bald patches of the scalp, ring-shaped lesions frequently develop on the face, neck and hands.

The colonies on Sabouraud's proof agar are acuminate, i.e., there is a conical peak projecting from the center of the colony. The peripheral portion of the colony is folded into ridges. The colony is of a creamy white color, the surface covered with a fine powdery coat. This is due to the abundant production of spores, which are round to pear-shaped, and borne laterally and at the tips of the filaments either with or without stalks. Chlamydospores are found in the vegetative mycelium.

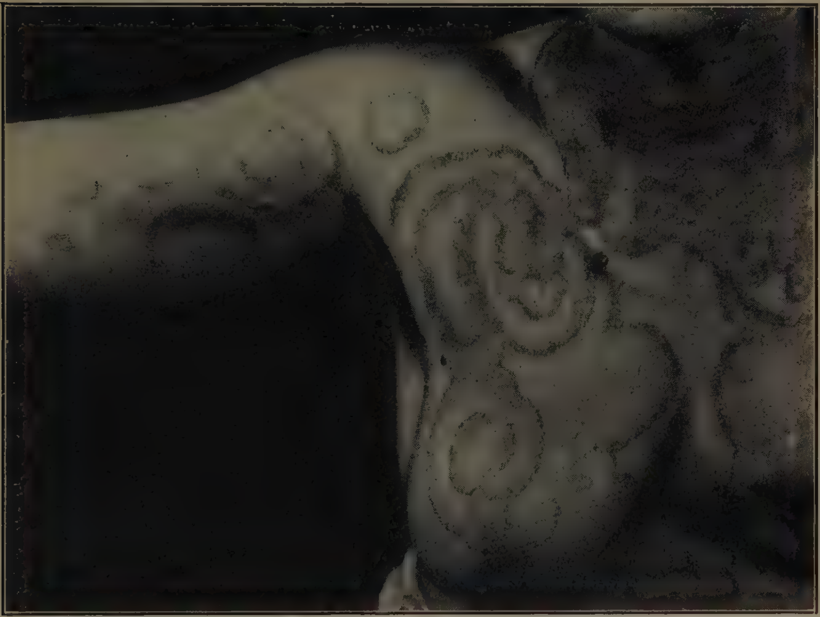

FIG. 65.—Ringworm of the smooth skin showing concentric rings, approaching the imbricate type.

Trichophyton crateriforme produces lesions of the scalp much as the above species, save that more of the hairs break off above the skin level, leaving stumps. The "spores" in the hair are more cylindrical than spherical, appearing quadrangular when viewed from the side. The chain of spores thus has the appearance of a ladder rather than of a string of beads, as in the preceding species. The colony is crateriform, i.e., sunken in the middle, with a raised ring at the periphery. In cultures the microscopic appearance is the same as *Tr. acuminatum.* A number of rarer closely related species are differentiated according to the color or texture of their colonies.

Trichophyton violaceum is more frequent in Russia, southeastern Europe and America, and produces lesions of the scalp of the same general character as the species just described. In the hair the spores are not so regularly in chains, and are rounded. The colonies are of the acuminate type, but when freshly isolated are of a deep violet color. This pigment is, however, soon lost on continued cultivation. Colorless sectors frequently appear in the pigmented colonies, together with sectors of transitional color. A number of varieties have been named according to these various pigmentations. Spores are not formed in cultures; the surface of the colony therefore remains smooth, i.e., without a powdery coat.

Tr. acuminatum and *Tr. crateriforme* can be successfully inoculated into guinea pigs, producing however only a localized lesion which soon heals. *Tr. violaceum* is not pathogenic to lower animals.

Neo-endothrix Trichophytons.—Neo-endothrix Trichophytons are transitional between the purely human Trichophytons and the Ectotrichophytons of animal origin, in that their mycelium appears in relation to the hair as does a pure endothrix form in the early stages of invasion, namely partly within and partly without the hairs. The infections produced are also transitional between those caused by the human and animal types of Trichophytons, showing some tendency to inflammatory reaction, but not so marked as in the Ectotrichophytoses. Infections by these transitional forms seem to be rare, save in Germany and Austria. Plaut notes that they are common in country districts, rare in cities.

There are two species, *Trichophyton cerebriforme* and *Trichophyton plicatile,* differentiated mainly on colony form. The former is more common. It produces ringworm of the scalp, beard, and the smooth skin, with varying degrees of inflammatory reaction. Plaut found it on cats. It may be successfully inoculated on guinea pigs. The cultures resemble *Tr. crateriforme,* but the surface of the colony is more folded or wrinkled.

Ectothrix Trichophytons.—Ectothrix Trichophytons are clinically sharply differentiated from the endothrix types by the more pronounced inflammatory reaction which they produce in the skin. This would seem to indicate a greater virulence. But these more acute types of trichophytosis tend to heal more rapidly than those with a less pronounced inflammation. Whereas with Microsporum and Trichophyton infections the skin changes are usually limited to congestion of the advancing border and scaling of the epidermis, with infections by the Ectotrichophytons there is also an exudation of serum and leucocytes into the skin, leading to more or less bogginess and infiltration of the deeper tissues. Not infrequently pus oozes in small drops from the mouths of the hair follicles, or distinct pustules may appear. In more acute forms certain areas of skin may become elevated and feel quite boggy when palpated, as though an abscess of some size were developing, pus oozing from all of the hair follicles when the skin is squeezed. Such a lesion is called a kerion. Suppurative lesions of the beard are referred to as "Sycosis."

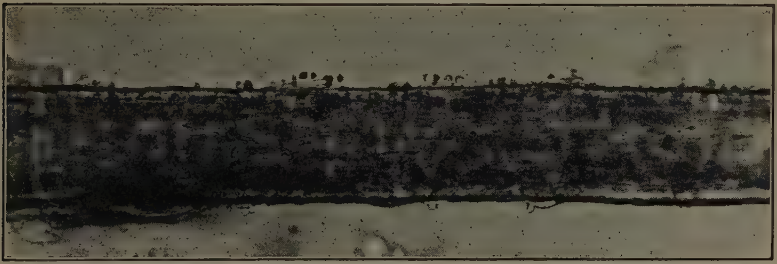

FIG. 66.—A hair from ringworm of the scalp. This is a small-spored Ectothrix Trichophyton. The spores are seen on the surface of the hair.

The Ectotrichophytons are divided into two groups, those with small spores in the lesions (about 3μ in diameter) and those with large spores (about 5–7μ in diameter). Species of the first group grow more rapidly in artificial cultures than those of the second.

The small-spored Ectotrichophytons may be confused with Microsporum if the differentiation is made solely on examination of the hairs, for they present the same general appearance—a few articulated hyphae within the hair and numerous spores on the surface, in its sheath. But these spores, unlike those of Microsporum, are produced by a fragmentation of filaments of mycelium and therefore tend to be arranged in chains rather than in an irregular mosaic. The spores of the two fungi are about of a size. But in microsporosis one never sees the inflammatory reaction with a tendency to deep infiltration of the skin characteristic of the small-spored ectotrichophytoses.

In adults the small-spored Ectotrichophytons usually cause infections of the smooth skin, especially the extensor surfaces of the fore-arms. In children lesions of the scalp, face and hands occur. The lesions are usually of the circular type characteristic of the ringworms.

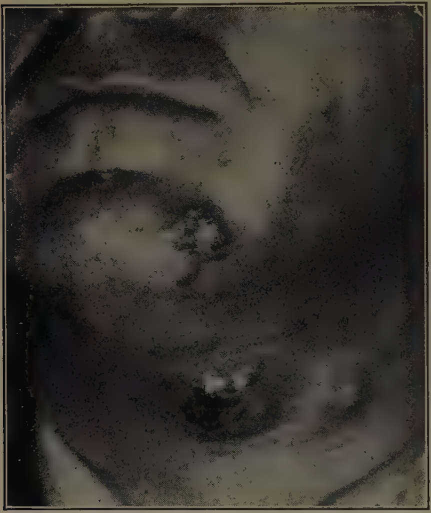

Fig. 67.—A suppurating ringworm of the beard ("sycosis"). This is due to an Ectothrix Trichophyton.

The small-spored group form white colonies of characteristic form, bundles of hyphae radiating from the periphery to give a star-like appearance to the colony. There are a number of species divided into two groups, the *gypseum* group and the *niveum* group. In the former the colonies are star-like, the surface powdery and "plaster-like"; in the latter they are "like freshly fallen snow," downy on the surface. In cultures of the *gypseum* group, in addition to the aleuriospores and spindle spores characteristic of the Trichophytons, there occur frequently peculiarly coiled strands of mycelium resembling the early stages of perithecial formation in organisms like Aspergillus. These, as well as the spindle spores, are lacking in the *niveum* group.

All of the members of this group will readily infect guinea pigs. They have been found causing infections of various domestic animals, especially horses, but also cats and dogs.

The large-spored Ectotrichophytons are also divided into two groups, those with downy colonies, and those with faviform (i.e., resembling *Achorion schoenleinii*) colonies. This difference is due to the amount of aerial mycelium, which is formed early and abundantly in the former, later and less abundantly in the latter. The colonies of the faviform group have the waxy appearance of those of the favus parasite. Cultures of the downy type produce conidia as the only fruiting bodies, whole those of the faviform type fail to produce these, forming only mycelium, and chlamydospores.

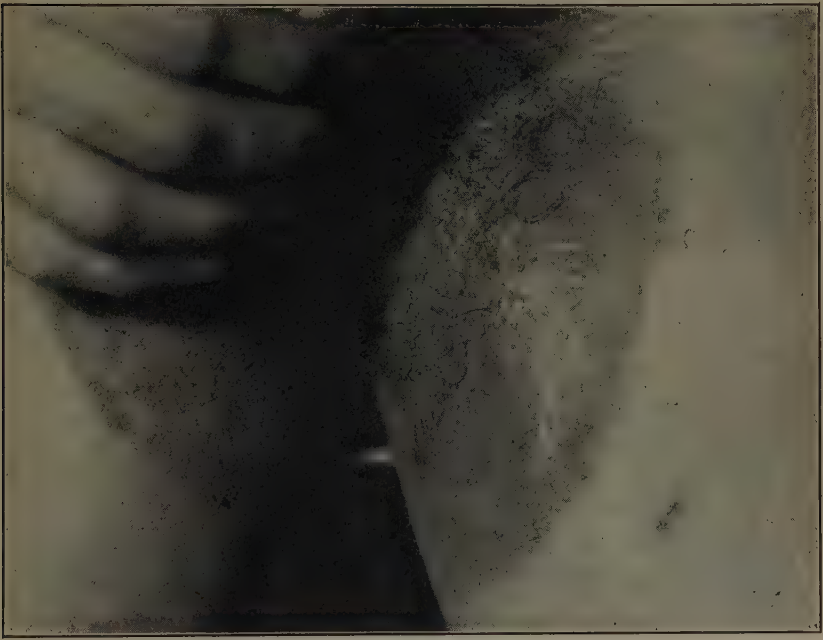

FIG. 68.—Tinea cruris, due to Epidermophyton.

All of the large-spored Ectotrichophytons may produce ringworms of the smooth skin, scalp, or beard. They occur more commonly in adults than children. They also occur on a variety of domestic animals. They may be successfully inoculated into guinea pigs. One species, *Ectotrichophyton rosaceum*, occurs frequently in various domestic birds.

Epidermophyton.—Members of the genus Epidermophyton cause an infection of the skin known as tinea cruris, or eczema marginatum. It occurs on the smooth skin in those parts which are likely to be moist, most frequently the inner surfaces of the thighs, but also in the axillae, the folds of the buttocks, or under the breasts. It is much more frequent in warm climates and is especially prevalent in India, where it is known as dhobie itch. The affection is somewhat different from the other ringworms, especially in the fact that it does not tend to heal in the center as it spreads at the periphery.

The parasite is found in scales of epidermis as articulated filaments of mycelium breaking up into chains of oval to round oidia.

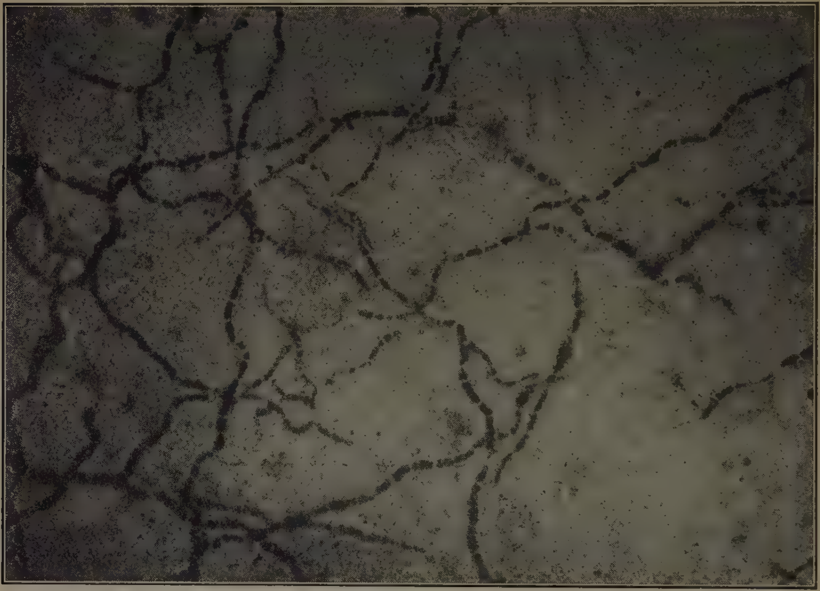

Fig. 69.—Filaments of Endodermophyton in a scale from Tinea imbricata. After Castellani.

In artificial cultures, reproduction takes place entirely by spindle spores, which are more abundant than in the trichophytons. No conidia are formed. Aside from one species found in monkeys, they are strictly human parasites and cannot be transmitted to lower animals. The most important species, *Epidermophyton cruris*, is identified by the greenish to lemon color of the colonies.

Endodermophyton.—The various species of Endodermophyton are the etiologic agents of a variety of ringworm occurring in China, the Malay peninsula and various Pacific islands, known as Tokelau ringworm or tinea imbricata. The concentric rings so characteristic of the tineas reach their highest development in this disease; there is much more scaling of the epithelium than in the other ringworms; as a result there is produced on the skin a complicated pattern of concentric white rings.

The organism grows within the epithelium between the superficial and deeper layers. It appears in the scales much as does Epidermophyton. Cultures are obtained with extreme difficulty. First cultures must be made in liquid media; after these grow, they may be transferred to agar. The colonies are at first like those of *Achorion schoenleinii*. No spores are formed in cultures.

Fungus Infections of the Hands and Feet.—During recent years a series of fungus infections of the hands, and more frequently the feet, have attracted considerable attention. The condition was undoubtedly overlooked for many years, but, on the other hand, there seems but little doubt that it has markedly increased in recent years. According to Mitchell,[7] this is due in part to the demobilization of a considerable number of soldiers at the end of the World War who were suffering from tinea cruris. But undoubtedly a greater factor has been the increased participation of the population in athletics and golf, the infection being contracted by going barefoot in dressing rooms and showers. The infection is extraordinarily prevalent among college students. Thus Legge, Bonar and Templeton[4] found the disease in 78 per cent of the men and 17 per cent of the women in a survey at the University of California. The much lower incidence in the women is attributed to the use of rubber bathing slippers.

The condition usually begins in the folds between the toes, but eventually tends to involve the sole. The lesions begin (frequently rather suddenly) as a series of small blisters, which tend to coalesce. This is followed by scaling and the development of eczema-like lesions. The condition may yield to treatment, but tends to recur, especially during the summer months. The presence of fungi has been demonstrated in the healthy skin some distance from the lesions, and in the apparently healthy skin between attacks.

A variety of fungi have been isolated. Although some of the cases have been definitely associated with tinea cruris, and *Epidermophyton cruris* has been isolated from a considerable number of cases (20 per cent in Mitchell's series), it is now apparent that the majority of cases are caused by various species of Trichophyton. Kaufmann-Wolff [3] described an organism which she found almost to the exclusion of other species, which has also been found the most common species by others who have made cultures (33 per cent of Mitchell's cases). This is a species of Trichophyton, apparently very similar to *Trichophyton equinum,* but without virulence for animals.

SAPROPHYTIC SKIN FUNGI

Pityriasis Versicolor.—Pityriasis versicolor is the most common of the dermatomycoses. It is characterized by a brownish discoloration (coffee-and-milk color) of the skin, occurring most frequently on the trunk, especially the chest, also in the bend of the elbow and knee, and inner surfaces of the thighs. It is accompanied by some scaling and a slight itchiness.

The causative organism, *Malassezia* (formerly Microsporon) *furfur,* may be found in large numbers in scales of the epidermis mounted in hydroxide solution. It appears as short irregular strands of branched mycelium accompanied by large numbers of round spores varying considerably in size. When stained with carbol fuchsin the spores are seen to contain several deeply stained bodies of globular form in a less deeply stained protoplasm. Their nature is unknown. The spores tend to be arranged in clusters. They are probably produced from the mycelium as conidia.

It is doubtful that this organism has been artificially cultivated. A number of investigators have claimed successful cultivation, but only occasionally after many trials. The cultures so obtained show only a few budding cells and sterile mycelium, nothing to indicate the systematic relationships of the organism. We have no data, therefore, by which we can classify this fungus. Castellani places it near Hormodendrum.

Erythrasma.—Erythrasma is an affection of the moist skin areas, not so common as pityriasis. It is characterized by a yellowish-brown discoloration. The fungus appears in the epidermal scales as very minute (about 1 micron in diameter) filaments of branched mycelium and chains of similarly minute spores. It is generally referred to as *Microsporon minutissimum,* but there seems now but little doubt that it belongs with the Actinomycetes.

Piedra.—Piedra, or Trichosporosis, is an affection of the hairs occurring in tropical climates. Masses of fungus mycelium grow as little hard knobs on the hairs. It appears that various different fungi may produce the condition in different parts of the world.

Thus, in South American Indians the disease occurs on the hair of the head or beard, and a fungus named *Trichosporum giganteum* is obtained, characterized by very large conidia. Here the disease is perhaps due to the use of a native mucilaginous hair dressing. In Ceylon, Castellani described a similar condition of the axillary and pubic hairs due to an Actinomycete associated with certain pigment-forming cocci.

Pinta.—Pinta is a disease characterized by pigmentation of the skin, occurring in Mexico, Central and South America. It occurs on the exposed parts of the skin, and is most prevalent in moist lowlands and during the rainy season. It occurs particularly frequently on the legs of workers in mines containing water rich in sulphates and other salts. A number of varieties are described, blue, violet, black, red, gray, and white. The white condition is due to a destruction of the natural pigment of the dark-skinned natives, and appears as the disease regresses. The other colors are due to different species of fungi. Aside from some scaling and itchiness, there are no other changes save the pigmentation. It is very prevalent among the lower native types in some of the tropical American countries.

A large number of different fungi have been isolated from cases of pinta, more than twenty in all, including species of Aspergillus, Penicillium, Monilia. According to Castellani, however, these are probably all accidental organisms having nothing to do with the disease. He found in cases of yellow pinta an organism related to the organism of pityriasis versicolor, namely *Malassezia tropica*, which produces the same disease known as tinea flava in India. The black and blue varieties are according to Castellani but areas of hyperpigmentation. They may not be due to fungi, or may be after effects of ringworms. In some cases of black pinta he could demonstrate a species of Cladosporium.

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CHAPTER VII

FUNGI TRANSITIONAL BETWEEN MOLDS AND YEASTS: OIDIUM AND MONILIA

We have seen that many diverse types of fungi may at times assume a unicellular form, frequently the single cells being hardly distinguishable from yeasts. Since these forms also produce other well-defined reproductive bodies, there is little question concerning their classification. But there exists quite a group of fungi which produce at times mycelium, at times free yeast-like cells, which do not form conidia or other bodies serving to identify them, or which may produce conidia that grade so imperceptibly into the free yeast-like cells that it is frequently difficult to tell which is which. Yeast-like cells are formed so regularly and comprise so prominent a part of the microscopic picture that, in spite of their non-specific characters, they are given considerable attention in classification. Such forms are looked upon as being transitional between the molds and the yeasts proper. Being transitional types, we find a great deal of confusion and contradiction regarding their proper nomenclature and classification. The same organism has been placed in half a dozen different genera depending upon the interpretation given by this author or that to the yeast-like cells. We must include in this transitional group an undoubtedly very heterogeneous mixture of fungi whose true systematic relationships are quite unknown.

The microorganisms to be considered have been mostly referred to the genera Oidium and Monilia, though many other generic names have been used. But these two genera are themselves rather poorly defined, and as used today they are given meanings rather different from their original definitions. Moreover, the two names have been used interchangeably, the organism of thrush, for instance being referred to in medical literature with about equal frequency as *Oidium albicans* and as *Monilia albicans*. One finds a wide variety of characters mentioned as bases for differentiating the genera, depending upon the nature of the experience of the author. Thus Castellani differentiates Oidium from Monilia by the production of gas from dextrose; Stevens by the invasion or lack of invasion of the tissues of host plants; Gilman and Abbott on the branching or lack of branching of the conidiophores.

There is, however, in recent literature a clear tendency to separate the two groups on the basis of the mode of production of free cells from the mycelium, reserving the name Oidium for those forms which give rise to free cells by a disarticulation of the mycelium itself, as occurs so characteristically in *Oidium lactis*, and referring to Monilia those species in which free cells arise by budding from the mycelium, as is found in the various pathogenic Moniliae of which the thrush parasite is an example. These two groups would thus correspond with the Arthrosporinae and the Blastosporinae, respectively, in Vuillemin's classification.

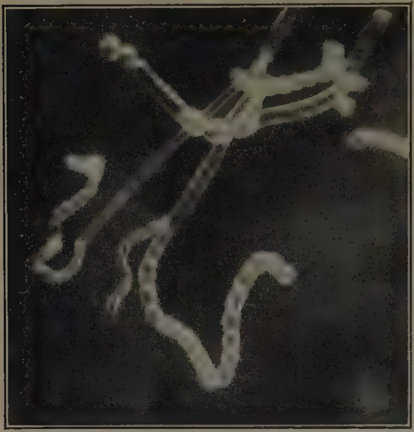

Fig. 70.—*Oidium lactis*, showing development of oidia. Photomicrograph by dark field illumination.

Oidium lactis.—This is a very common and widespread mold, extraordinarily resistant to heat and antiseptics, which grows everywhere where lactic acid is present—on sour milk, cheeses, butter, sauerkraut, silage and pickles. It is said to oxidize lactic acid completely to carbon dioxide and water, thus reducing the acidity of the medium in which it is growing. This, however, has not been satisfactorily proved, for it is also actively proteolytic, producing ammonia from organic nitrogenous compounds, and the reduction of acidity of sour milk may well be due to neutralization by the ammonia rather than oxidation of the lactic acid.

It is of practical importance in the dairy industries, for because of its proteolysis it gives rise to flavors and odors which are very undesirable (except perhaps in Limburger cheese, in the ripening of which it probably plays a part). It is especially troublesome in butter. It grows, however, only in the presence of lactic acid. The presence of this organism in any abundance in dairy products is a good indication of uncleanliness, since it gives evidence that vessels have been used without proper cleansing or sterilizing after milk has stood in them long enough to sour.

The organism is easily recognized. On milk or other products, as in artificial culture media, it produces a rather firm, felt-like mass, pure white in color. On solid media this is rather firmly adherent. In older cultures there is considerable aerial mycelium. Microscopic examination shows both segmented, branched mycelium and numerous large, free, square-ended oidia, the latter frequently joined together in short chains at alternate corners in zigzag arrangement. The aerial hyphae bear chains of oval conidia.

Blastomycosis.—The name generally given to this peculiarly American disease is doubly a misnomer. It was applied first under the mistaken impression that the causative organism is a true yeast, and second that "Blastomyces" is the proper scientific name for the yeasts. The organism is not a true yeast, although it presents the yeast-like form in the tissues; and the name "Blastomyces" has been applied to one of the other Fungi Imperfecti, the proper scientific name for yeasts being Saccharomyces.

The disease has been confused with infections by true yeasts or by Monilias of the type of the thrush parasite. The organism is quite different from these, however, in the tough character of its growth on solid media which is not at all like the pasty growth of the yeasts and Moniliae, and by the abundant production of aerial mycelium in cultures. It has also been confused with coccidioidal granuloma which it may resemble closely in its clinical features, but the organism is differentiated by the fact that it reproduces in the tissues entirely by budding, never by endogenous spore formation.

The disease was first discovered in Baltimore (1894) by Gilchrist. It has since been reported from all parts of North America, but occurs most frequently in the central states.

Gilchrist named the organism *Blastomyces dermatitidis.* The organism was extensively studied by Ricketts,[37] whose monograph first showed clearly the distinctive characters by which this organism may be differentiated from the yeasts, although he failed to distinguish it clearly from some of the other yeast-like fungi. He proposed to place the organism in the genus Oidium, and to name the disease oidiomycosis. The organism has also been termed *Cryptococcus gilchristi* by Vuillemin, which terminology is followed by Sartory and by Castellani; but such a classification places an undue emphasis on the yeast-like appearance of the organism in the tissues. It has been placed in the genus Mycoderma by Brumpt.[8] This is the equivalent of Rickett's position, since Brumpt's Mycoderma is the equivalent of Oidium as defined above. Since reproduction by disarticulation of the mycelium is a prominent character in artificial cultures, the author will in this book follow the proposal of Ricketts and include the organism in the genus Oidium, as *Oidium dermatitidis.*

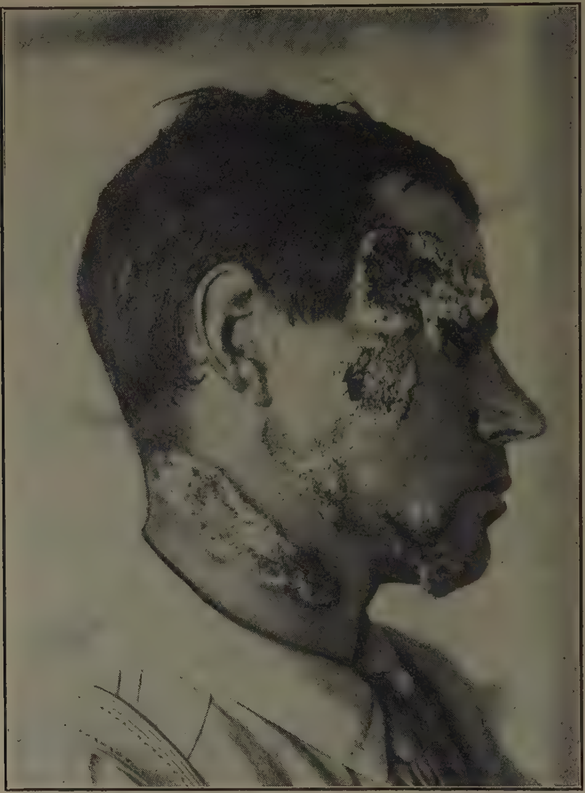

FIG. 71.—Blastomycosis.

In the majority of cases the disease occurs primarily in the skin and subcutaneous tissues as a wound infection. A number of cases primary in the lungs have also been reported. It may be that the parasite grows upon vegetable matter, as in the case of Sporotrichosis, but this has not been proved. A very large proportion of the published cases have been farmers. At least one case of direct transmission (autopsy infection) is known.

Pathology.—The primary skin lesions are quite characteristic in appearance. Beginning as a small, firm papule, a number of secondary nodules develop about this which gradually enlarge and coalesce. These break down in the center and discharge pus through a number of small fistulae. As the disease progresses, there gradually develops a large, elevated mass of tissue with an irregular ulcerated surface that resembles somewhat a breaking down cancer, sometimes a tuberculous ulcer. Slight pressure on the mass will cause pus to ooze from a number of minute openings.

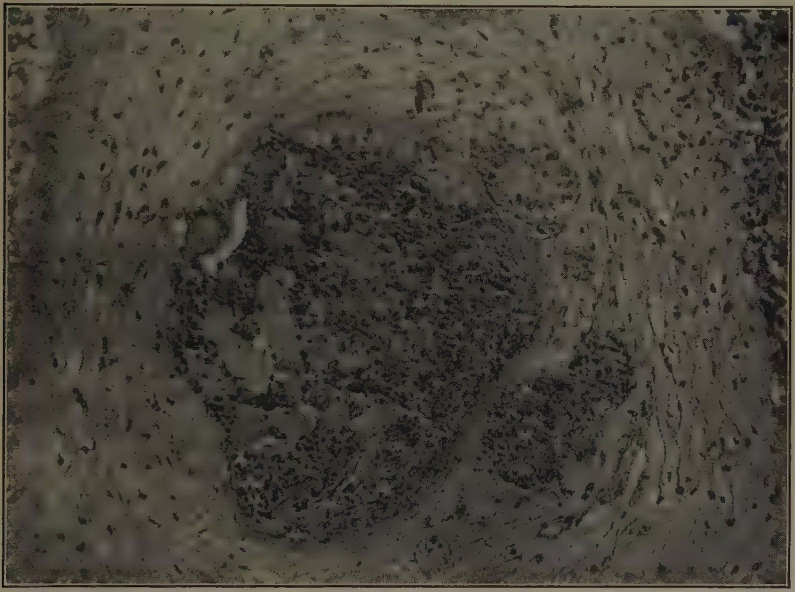

FIG. 72.—Section through the skin in a case of blastomycosis, showing a characteristic intraepithelial abscess.

Even the microscopic appearance of the tissue presents some resemblance to both tuberculosis and cancer. For the inflammatory reaction, particularly in the subcutaneous fibrous tissue, is largely granulomatous in nature, there being much new-formed connective tissue and considerable infiltration with mononuclear leucocytes; even giant cells may be formed, while the epithelial tissue, in response to the irritation, undergoes considerable proliferation and may send long, finger-like processes down into the inflammatory tissue, much as in an epithelioma. A very constant and characteristic feature of the microscopic pathology is the occurrence of minute abscesses, i.e., spaces filled with polymorphonuclear leucocytes, in the epithelium proper. In these miliary abscesses the parasites are found.

The disease tends to progress slowly, spreading through the subcutaneous tissues, and may give rise to new lesions at a distance, by contact, by way of the lymph channels or by way of the blood stream. Dissemination through the lymph channels does not occur so regularly as in sporotrichosis.

Primary blastomycosis of the lungs frequently bears a striking resemblance to pulmonary tuberculosis in its course and symptoms, and is often so mistakenly diagnosed, the first indication of the true nature of the disease being a rather sudden generalization of the infection with the development of subcutaneous abscesses.

Dissemination by way of the bloodstream leads to a development of multiple abscesses throughout the body. These are particularly prone to occur in the subcutaneous tissues, but may also develop in the muscles, under the periosteum of the bones, or in internal viscera. The subcutaneous abscesses are quite characteristic and quite different from the primary skin lesions. They develop painlessly and without much local heat or redness; they are soft and fluctuate, and when opened evacuate a considerable amount of pus, from which the organism may be easily cultivated. The generalized form of the disease is accompanied by a septic type of fever curve and is probably always fatal.

Diagnosis.—The diagnosis is established by finding the organisms in the pus. This is relatively easy. In the body tissues the organism occurs only as a round or oval yeast-like cell reproducing by budding. The cell wall is rather thick and highly refractile appearing as a bright line bordered by two fine dark lines ("doubly-contoured") in unstained preparations. Only one bud is formed at a time; the cell wall of the bud is much thinner than that of the parent cell. The protoplasm is granular and almost invariably contains one or more rather refractile vacuoles.

The organisms are best sought in wet preparations prepared with 20 per cent NaOH, examined with the high, dry lens. The finding of doubly-contoured cells a trifle smaller than the leucocytes, with granular contents is very suggestive. If these show distinct budding the diagnosis is practically certain. Air bubbles and fat droplets may be mistaken if the granular contents are not looked for.

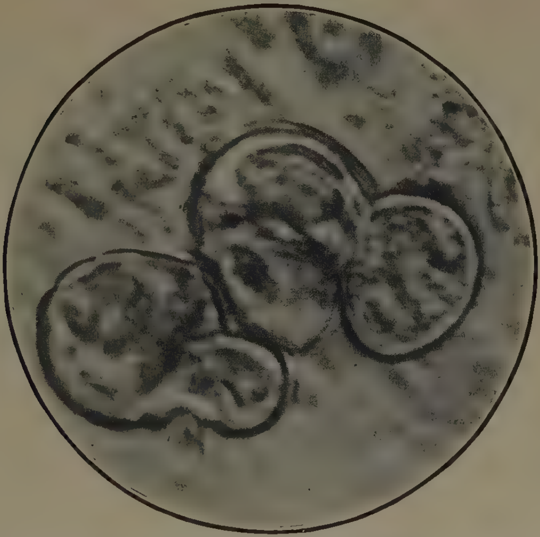

Fig. 73.—Budding yeast-like cells of *Oidium dermatitidis*, in a wet preparation of pus from a case of blastomycosis.

Cultures.—Cultures are readily obtained on Sabouraud agar if there are not too many contaminating bacteria. The organism will not grow on dextrose tartaric agar.

The appearance of cultures on agar is quite variable, depending much on the age of the culture, the number of transfers since the original isolation, and the rapidity of transfer. In general three types of cultures may be recognized, though there will be seen many transitions between them. The type of the culture depends on the degree to which the organism assumes the unicellular or the mycelial form.

The first type of growth may be designated the "mealy" type. The first cultures from lesions generally assume this form. The growth resembles somewhat that of the tubercle bacillus, forming a dry, somewhat wrinkled growth on agar which is friable and breaks up into small fragments when removed with the inoculating wire. When examined under the microscope, this is found to consist of forms transitional between the unicellular form and mycelium. There will be found some round budding cells similar to those occurring in the lesions, but for the most part the cells are oval to cylindrical and tend to form articulated chains of cells which may branch—the beginnings of mycelium. Subsequent subcultures will nearly always begin as this type of growth, even though made from cultures presenting an abundance of mycelium.

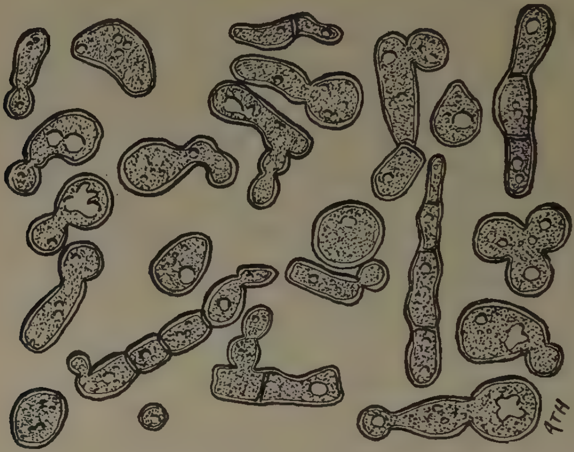

FIG. 74.—*Oidium dermatitidis* in a young culture, showing transitions from the yeast-like form to mycelium.

The second type of growth may develop directly from the first in the same culture upon aging, but more frequently does not appear until after one or two subcultures. It is characterized by the production of a number of minute prickly elevations on the surface of the growth, at first rather solid, later becoming loose and rather cottony at the tips, and thus gradually transforming to the third type. The prickles are made up of closely packed together filaments of true mycelium, and are to be looked upon as abortive coremia.

The third type is the cottony or woolly form of growth, characterized by an abundance of loose-meshed aerial mycelium. In this type the one-celled forms have completely disappeared, and both the submersed and aerial portions of the growth appear as narrow branched filaments of typical septate mycelium.

The woolly type occurs regularly in old laboratory strains which are frequently subcultured. If they are allowed to go for some months without transferring, they will frequently revert to the mealy type of growth for a time. The appearance of the coremium-like structures giving the prickly appearance to the surface may entirely fail to develop with some strains, and is seldom seen after the first few subcultivations.

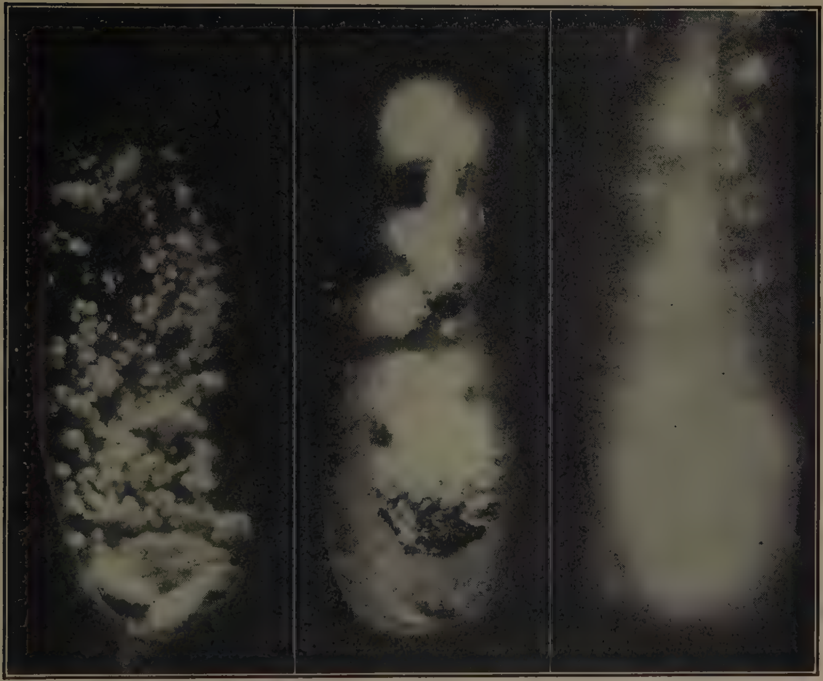

FIG. 75.—Cultures of *Oidium dermatitidis*. Left, the mealy type. Center, the prickly type. Right, the woolly type.

Another type of growth is sometimes seen in first cultures, particularly in Petri dishes. The organisms give rise to round, elevated colonies, somewhat conical in profile, which are smooth and glistening, firm and tenacious. These are made up entirely of closely packed together filaments of septate mycelium. Only after some days or weeks does aerial mycelium develop. In this type of culture the yeast-like cells of the lesions become transformed directly into mycelium, without first undergoing a stage of pseudomycelium as occurs in the mealy type of growth.

Spring [39] describes a tan to brown color developing in the aerial mycelium. Mellon [34] noted a dark pigmented variant in his study of pleomorphism of this organism, and Michelson,[35] who has rather extensively studied the cultural variations on different media also records some dark brown strains. In an experience limited to about a dozen isolations, the author has never seen such pigmented cultures.

Spores.—Spring [39] has recently described the appearance of conidia on the aerial mycelium. This is the first published report of the occurrence of such reproductive bodies in the organism of blastomycosis, so far as the author knows; he has found them in one of three strains examined since the publication of that paper. The appearance of the conidia is made manifest by a somewhat powdery appearance of the superficial mycelium. The conidia appear singly on short stalks given off at regular intervals along the filaments of aerial mycelium, and are globular in form. Their arrangement is somewhat like that in Sporotrichum. In one strain studied by Spring, the conidia appeared in short chains, resembling the arrangement in Scopulariopsis. Mellon [34] has described the occurrence of ascospores in one strain and has attempted to explain on that basis the pleomorphism of the organism. When these more recent findings have been verified and further studied, we will probably have to reclassify this organism.

Animal Inoculations.—Animal inoculations are not uniformly successful and cannot be relied upon for diagnosis. Spring [39] found that mice are the most susceptible of the common laboratory animals, guinea pigs being more resistant, and rabbits practically immune. Inoculated intraperitoneally, small caseous nodules develop on the peritoneal surfaces, which contain the budding yeast-like cells. These are more numerous in mice than in other animals. The type of tissue reaction in experimentally inoculated animals varies, with the virulence of the strain and the resistance of the animal, from frank abscesses to lesions like tubercles, including giant cells.

Immunologic investigations have on the whole proved negative. A protective immunity cannot be established in animals. Cutaneous reactions with extracts of cultures ("blastomycetin") are lacking in patients affected with the disease; on the other hand, it has been possible to demonstrate a specific sensitization in experimentally inoculated animals by intratesticular injection of culture extracts. Positive complement fixation and agglutination reactions have been reported.

The prognosis of blastomycosis is not good, especially in pulmonary cases. A rather large proportion of cases terminate fatally by generalization of the infection through the blood. Although undoubtedly a great many cases have been cured by internal administration of iodides, the results with this drug are not nearly so striking as in sporotrichosis. Michelson found that some inhibition of growth was apparent with 1 per cent potassium iodide added to cultures, but growth was not completely inhibited until 8 per cent was used, a concentration which is of course impossible in the body. Copper salts have been frequently used as local applications, and gentian violet has also been recommended. Spring, however, points out that the parasite will resist the action of this dye in cultures, in concentrations far higher than can be obtained in the body tissues.

Chromoblastomycosis.—Although the parasite of this disease apparently belongs elsewhere in the classification of the fungi, the disease and the appearance of the organism in the tissues seem to resemble blastomycosis so closely that it may well be briefly mentioned here.

The disease is quite rare, about thirty cases being on record. The great majority of cases have been observed in South America. It is localized most frequently on the legs, having its origin in wounds, a number of cases being individuals who habitually went barefoot. The infection tends to remain localized to an extremity. The lesions are of a granulomatous nature, resembling those of blastomycosis, including the production of intraepithelial abscesses.

The organism was first described by Medlar.[33] In the tissues two types of cells occur, large sclerotic cells with a thick brownish black wall, and smaller budding cells. The sclerotic cells may divide, forming an aggregation of such large thick-walled cells, which Medlar considers to be a sclerotium. The budding cells may be formed from the sclerotia. They may gradually enlarge and become sclerotic cells. Similar sclerotic cells are formed on media containing serum, but in all media a development of true mycelium occurs, and on solid media aerial mycelium develops which produces conidia. These occur in clusters at the tips of the conidiophores. The colonies are quite dark, brown to black, in color. Medlar named the organism *Phialophora verrucosa*, placing it in a new genus of the Dematiaceae. The disease is known variously as dermatitis verrucosa and as chromoblastomycosis.[11]

Coccidioidal Granuloma.—This disease is quite rare, about 200 cases being on record, but owing to its quite limited geographic distribution and high mortality, is quite important where it occurs. The disease bears some resemblance to blastomycosis (though more severe), and the parasite itself superficially resembles the organism of blastomycosis in that it presents itself in the tissues as doubly-contoured cells. But it differs markedly from the organism of blastomycosis in that *it never reproduces by budding*, and from all other pathogenic fungi in that in the tissues it multiplies entirely by endogenous spore formation. The systematic position of the organism is quite undetermined. Several authors have looked upon the intracellular spores as ascospores and have placed the organism provisionally in the Ascomycetes. But these spores are much more numerous than any ascospores, and there has been found no trace of sexuality in their production. The spores are very similar to those formed by some of the lower Phycomycetes, and Brumpt classifies the organism with the Chytridiales of the Archimycetes. This seems more plausible than the former view, but the characteristic septate mycelium formed in cultures is quite foreign to that group. From a practical standpoint, it is well to consider this organism along with the parasite of blastomycosis.

This disease shows the most striking limited geographic distribution of all the mycoses. A few cases have been reported from Brazil, and scattered cases from various parts of the United States, but the great majority (80 per cent) have had their origin in the San Joaquin valley in California. The reason for this limited distribution is not known. The disease has also been found in cattle and sheep in this area. The great majority of cases have been adult males.

The disease may be primary in the lungs or skin, as in blastomycosis; but pulmonary cases are the more frequent, and some of the cutaneous cases are secondary to lung involvement. The cutaneous type resembles blastomycosis very closely. The pulmonary type bears a striking resemblance to tuberculosis. The lesions also resemble tubercles very much, to such an extent that at times a differentiation is almost impossible. The disease is much more severe than blastomycosis, running a more acute course with fever, with a greater tendency to become disseminated by the blood-stream; it is almost invariably fatal. Although a few cases have been reported as apparently cured by the use of colloidal copper and of tartar emetic, practically the only cases which have recovered have been those in which the lesions were limited to an extremity that was amputated.

Morphology.—The parasite is found in the body tissues, pus or sputum as a round unicellular organism, varying greatly in size (from 5 to 50μ). The larger cells have a thick cell wall; in the smaller ones the wall is thin. As the cells increase in size, the protoplasm gradually becomes separated into a large number of small round cells, each of which develops its own cell wall, thus forming spores. On maturity the mother cell ruptures and the large number of small cells is liberated into the tissues, to gradually increase in size and go through the same process.

It is probably this mechanism by which enormous numbers of small cells are liberated into the tissues which makes the disease so extraordinarily malignant. In one case it was possible to isolate the organism by blood culture. The more acute forms present some of the characters of a septicemia.

Cultivated on dextrose agar, the round cells "germinate" and give rise to mycelium, septate and branched. An abundance of white aerial mycelium is formed, without conidia. In artificial cultures the organism bears considerable resemblance to the parasite of blastomycosis as far as gross appearance is concerned, but differs microscopically in the abundant production of chlamydospores.

Animal inoculations are much more uniformly successful than with the organism of blastomycosis, both rabbits and guinea pigs being susceptible. When mycelium from cultures is inoculated intraperitoneally into guinea pigs, the individual cells of the mycelium become rounded and then separate from each other, whole chains of cells thus being transformed into free round cells. These then grow and proceed to develop intracellular spores (MacNeal and Taylor [30]).

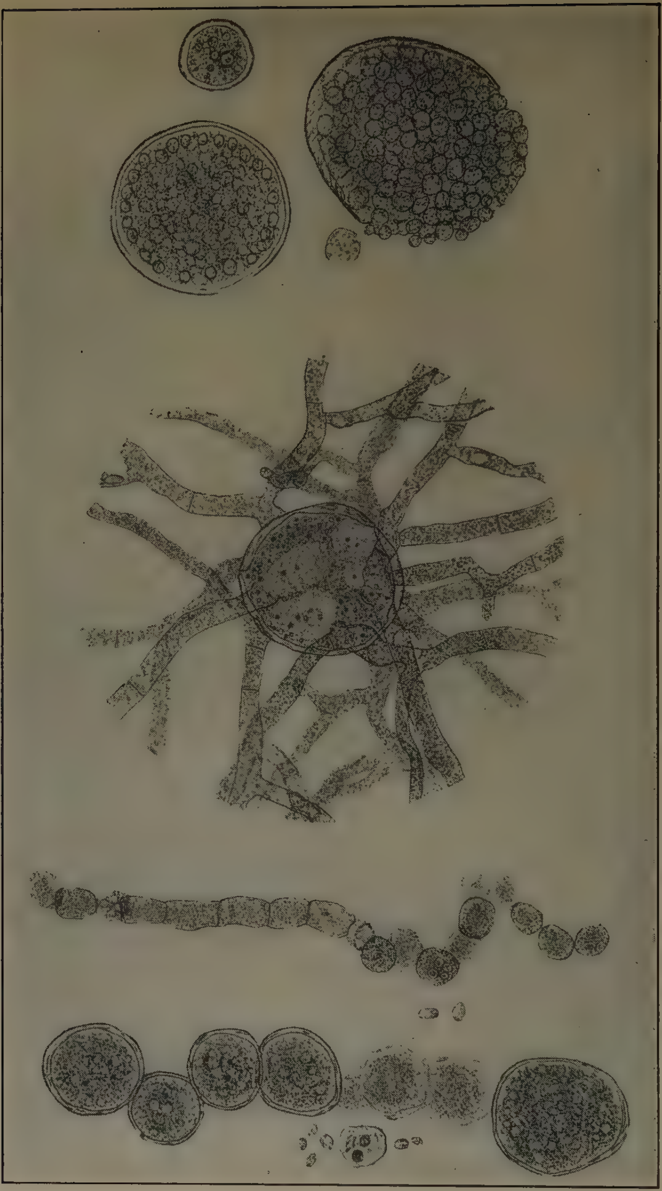

FIG. 76.—*Coccidioides immitis.* Above, developmental cycle in the infected tissues, and in anaerobic cultures. Note the large numbers of endogenous spores. Center, development of mycelium from one of the round cells, in artificial culture. Below, origin of round cells from mycelium in experimentally inoculated animal. After MacNeal and Taylor.

Thus the parasite presents two distinct modes of growth. In the animal body it is unicellular and multiplies by internally formed reproductive bodies. In artificial cultures it forms a multicellular mycelium, the only reproductive bodies being chlamydospores. It is interesting to note that MacNeal and Taylor obtained a development of the unicellular, endosporogenous type of growth in artificial media containing serum or tissue under semi-anaerobic conditions.

The appearance of the organism in the tissues, and particularly its mode of multiplication there, bears a close resemblance to the protozoan parasite, Coccidium, and the organism was first mistaken (and named) as such. Later its fungous nature was revealed by cultivation and inoculation. The name of the organism was then changed to *Coccidioides immitis.* This name seems to be generally accepted.

The Moniliae.—The term Monilia has at first glance quite distinct meanings to the general mycologist and the medical mycologist. To the plant pathologist the name at once suggests the nonsexual multiplication of the ascomycetous parasite of plums and other fruits, Sclerotinia. Here we have to deal with an essentially mold-like organism producing no yeast cells, which forms conidia in chains (sometimes branched) on aerial conidiophores, the individual spores connected by characteristic small intervening cells known as disjunctors. To the medical mycologist the term means an essentially yeast-like organism occurring on mucous membranes or skin lesions, which forms mycelium in semi-anaerobic cultures. There is no apparent similarity between such widely diverse forms.

And yet transitions between these types may be traced. The submersed mycelium of *Monilia albicans* in semi-anaerobic cultures gives rise to yeast-like cells by budding from the sides or tips of the filaments; these in turn form new round cells by budding, and so there are formed chains of cells; if one of the cells in the chain forms two buds, a branched chain of cells is produced. Now just such branched chains of cells are formed by the aerial mycelium of such a mold as *Monilia sitophila.* In the former case the cells budded off are capable of immediate multiplication. Being formed by the submersed mycelium, they are moist and not highly heat-resistant. They are vegetative cells rather than conidia. In the latter case the cells are true conidia. They are formed by aerial mycelium, are dry, heat-resistant, and equipped to remain dormant for some time. Thus the distinction is more physiological than morphological. Their mode of production is very similar.

It would seem, however, that the two types of Moniliae as known by the plant and human pathologists respectively are too widely different to warrant including them in the same genus. Unfortunately the name Monilia seems to be firmly established with both groups of workers. It would be highly desirable if some other name could come into general use for the yeast-like pathogenic fungi of thrush and related diseases. The name Parasaccharomyces, introduced by de Beurmann and Gougerot, has been redefined by Anderson to clearly comprise this group. It has the advantage of indicating the close similarity to the yeasts.

Monilia sitophila.—*Monilia sitophila* is a rather frequent laboratory pest. The very light and quite resistant conidia are formed very abundantly, and once distributed through a laboratory will reappear frequently as contaminants in plate cultures.

This organism does not typically form yeast-like cells but is a Monilia of the type forming conidia on aerial mycelium. The growth in test-tube cultures is quite characteristic. A very loose meshwork of aerial mycelium develops which nearly fills the tube. Conidia are formed in great abundance near the top of the tube, forming an orange to salmon-red colored powder. They are oval in form, and are produced as in Hormodendrum, by a budding process, the younger conidia arising from the older ones rather than from the conidiophore directly; thus branched chains of conidia are formed (Fig. 77).

Although Went [47] observed what he considered abortive perithecia some years ago, it was only quite recently that the perfect form of this fungus was discovered by Shear and Dodge.[38] The perithecia are black and flask-shaped, the asci are cylindrical, and contain elliptical dark ascospores (Fig. 78). Shear and Dodge created a new genus for this fungus, Neurospora, and recognize four closely related species. The organism is heterothallous, i.e., asci develop only after the fusion of mycelium from two separate plants. The ascospores germinate only after heating.

Monilia sitophila is most important as a cause of trouble in bakeries, giving rise occasionally to epidemics of infected bread. The spores apparently come from the flour. They are killed, when wet, at 72° in five minutes, but require 120° for thirty minutes to kill them when dry. As the spores are not easily wetted, and as the temperature of the interior of a loaf may not rise to 100° C. during baking, it is quite possible for the spores to remain alive after the loaf has been baked. The organism is also a cause of decomposition of stored bagasse. It is found growing on charred wood.

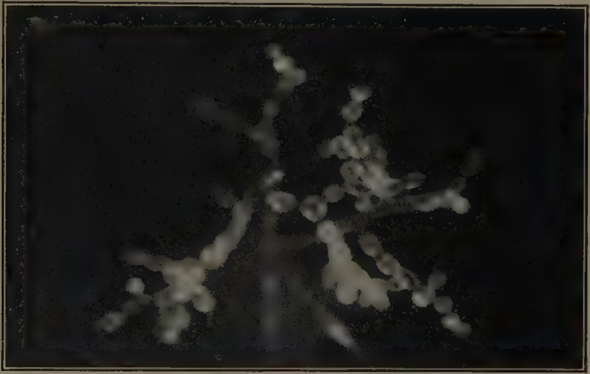

Fig. 77.—*Monilia* (=*Neurospora*) *sitophila*, showing arrangement of conidia. Photomicrograph by dark field illumination.

Went [47] has described the use of this mold in Java to produce a fermented food product from peanuts. The ground nuts are inoculated with the mold and pressed into cakes which develop a bright orange color after the fungus has grown. The same author [48] has investigated the enzymes and found it truly omnivorous, producing: maltoglucase, trehalase, raffinase, invertase, cytase, diastase, lipase, tyrosinase, rennin and trypsin. It also produces a slight alcoholic fermentation.

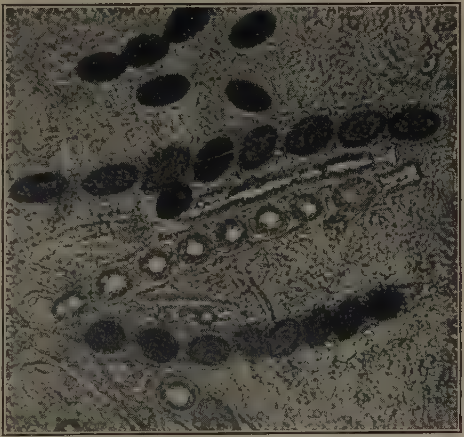

Fig. 78.—*Monilia* (=*Neurospora*) *sitophila*. Ascospores.

Monilia nigra and the Black Yeasts.—There have been described from time to time a series of yeasts or yeast-like organisms which produce a characteristic black color. They have been named variously, as *Saccharomyces niger*, *Torula nigra*, *Schizosaccharomyces niger*, and *Monilia nigra.* They have been isolated from a variety of substrates, mostly dairy products. Burri and Staub,[9] and more recently Maurizio and Staub [32] have found organisms of this type producing outbreaks of black spots in Emmenthaler cheese. Browne [7] reported an apparently identical organism as a cause of spoilage of raw sugar.

There has been some question regarding the identity of the various forms described, and their proper classification. The author has examined a dozen or more strains of so-called black yeasts from various sources, and though each strain might be fitted to the description of one organism or another in certain stages of development, they all showed marked transformations of the same general character on continued cultivation, so that he is not convinced that there is more than one species.

These morphologic transformations have been very completely described and illustrated by Maurizio and Staub. When first isolated the organisms grow as soft, pasty, yeast-like cultures, at first a pale yellow color but rapidly becoming a dark greenish-black. Examined at this stage, they show only oval budding yeast cells. If pour-plate cultures are made, the deep colonies, at first lenticular, will eventually sprout out numerous radiating filaments of mycelium that develop lateral clusters and chains of budding cells exactly as in cultures of *Monilia albicans.* In later subcultures mycelium also develops under aerobic conditions, the growth becoming tougher in consistency, and eventually producing an olive-green aerial mycelium which gives rise to branched chains of conidia.

The aerial conidial apparatus closely resembles that of Hormodendrum, the color also being the same. Hansen was of the opinion that these black yeasts are but yeast-like growth forms of Dematiaceous molds of the type of Hormodendrum, and this view was also supported by Lindner. The present writer also concurs in this opinion. Several of the strains which he has examined have produced, at times from the pasty yeast-like growth, at times from the aerial mycelium, characteristic two-celled conidia exactly like those of Cladosporium. This is probably the explanation for the use of the generic name Schizosaccharomyces by Marpmann.

Monilia nigra may be considered as a form transitional between the two types of Moniliae discussed above, for it forms budding vegetative cells in clusters or chains from the submersed mycelium, like the pathogenic Moniliae to be described below, and branched chains of conidia (produced also by budding) from the aerial mycelium, as in *Monilia sitophila.*

Moniliae without Aerial Mycelium.—The species of Monilia which remain to be considered differ from those just described in that *they never form aerial mycelium.* They exist either as free budding yeast-like cells, or may form mycelium, but the latter always remains submersed in the medium and gives rise to new yeast-like cells by budding. Although recent studies indicate the existence of a plurality of strains or species that may be differentiated by physiological characters, they are all remarkably alike in morphology; and if numerous species exist, at least they form a remarkably compact and homogeneous group. One species is a saprophyte, the remaining forms are known only from the human body, either as harmless parasites or as pathogens.

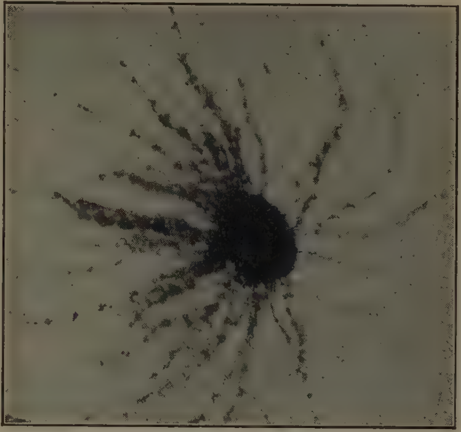

FIG. 79.—*Monilia nigra.* Deep colony, showing production of mycelium.

Morphology.—In all forms the yeast cell is the predominant growth form, mycelium being produced only exceptionally and under particular conditions. Grown on dextrose agar slants, for instance, there occurs a creamy white growth of pasty consistency and yeast-like odor. Microscopic examination of such a culture shows only round or oval budding cells which cannot be distinguished from true yeasts. In old agar slant cultures, however, one may find the growth dipping down into the agar in the form of narrow bands or fine filaments; and microscopic examination of this part of the culture will reveal, in addition to the budding yeast cells, some filaments of true, segmented mycelium. The formation of this mycelium may be better demonstrated in gelatine stab cultures. At the point of puncture there appears a heaped-up colony of buttery consistency, composed entirely of yeast cells. But after a few days there develop along the course of the stab many fine tufts of mycelium that radiate into the gelatin at right angles to the line of the stab, giving the growth a characteristic fir-tree appearance. If the tube is cut and microscopic preparations are made from these lateral projections, they will be found to be composed of mycelium, from which yeast cells are budded off in clusters along the sides and at the tips (Fig. 80). Such a gelatine stab culture is the best method for differentiating these Moniliae from true yeasts.

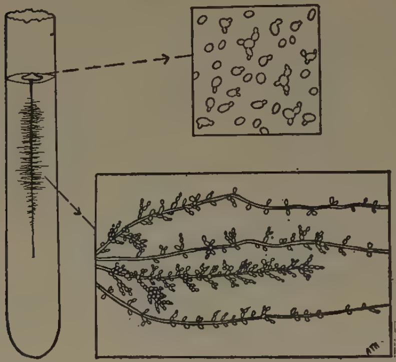

Fig. 80.—Diagram illustrating the characteristics of the pathogenic Moniliae. In a gelatine stab culture, only yeast-like cells are found at the surface, but filaments of mycelium radiate from the depths of the stab; these give rise to yeast-like cells by budding.

In agar pour-plate cultures the surface colonies appear exactly like yeast colonies, and contain only single budding cells. The deep colonies are usually at first lens-shaped and contain only yeast cells, but after a few days send out fine streamers of mycelium which give them a characteristic star-like appearance.

In liquid media, growth is restricted to the bottom of the tube, at least with most species. Here one may find either free cells alone, or some mycelium in addition, depending on the composition of the medium and the age of the culture.

In lesions produced by these organisms both mycelium and yeast-like cells will be found.

Causes of Pleomorphism.—There has been considerable speculation and investigation concerning the factors which determine the morphology of these organisms, i.e., as to when they will form mycelium and when assume the yeast form. Such investigations have been carried on almost entirely with the organism of thrush, but the results will undoubtedly be found applicable to other members of this group as well.

It was early found that aeration is a factor of prime importance, as is indicated in the stab cultures described above. On the surface of agar slant cultures no mycelium is formed. In liquid media, some filaments may be found, the degree of mycelium formation varying with the composition of the medium. Fineman [20] found that mycelium would form on the surface of agar slant cultures if these were incubated in an atmosphere of carbon dioxide.

Linossier and Roux [28] cultivated the thrush parasite in a "synthetic" liquid medium to which they added various carbohydrates, and noted that with the simple hexose sugars no mycelium was formed, whereas with the di- and polysaccharides they obtained abundant mycelium. They attempted from these observations to establish a hypothesis that "the complexity of form of the thrush parasite is proportional to the molecular weight of the food elements of the medium." Their observations were confirmed by Hickel and to a limited extent by Fineman. The latter author found that this effect of carbohydrates was true only in liquid media, yeast forms alone appearing on agar slants of the same composition as the liquid media used. In liquid media, however, mycelium was abundant if dextrin was the source of carbon, whereas only yeast forms appeared when dextrose was used. However, mycelium was equally as abundant in cultures containing galactose as in those containing dextrin. Now galactose is no more complex than dextrose, but is not so easily utilized for food. Fineman also found that decreasing the surface tension of the dextrin solution by adding sodium ricinoleate gave a pure yeast growth without mycelium. It would appear, therefore, that a multiplicity of factors may influence the morphology of this organism.

In general, however, it would seem that the form of the organism is more or less an expression of the rate of growth. Where conditions are favorable for rapid multiplication, as with an easily assimilable or fermentable carbohydrate and abundant aeration, the yeast form predominates. When conditions are not so favorable, mycelium is formed.

Spores.—The yeast cells budded from the mycelium in semi-anaerobic cultures are referred to as conidia by some authors. In addition chlamydospores, both intercalary and terminal, are occasionally formed. Fischer and Brebeck,[21] and also Vuillemin,[43] recorded observations of ascospores, and on this basis some authors have included these organisms in the ascomycetous genus Endomyces. But these observations, made long ago, have never been confirmed, although the Moniliae have been very intensively studied in recent years. There have also been noted round or oval bodies formed within the mycelium, that have been designated "endospores." These are probably fat vacuoles.

Differentiation of Species.—Although attempts have been made to differentiate the various species of Monilia on the basis of morphology, as size and form of the yeast-like cells, these characters are not sufficiently different to be used for such a purpose. Most of the species are also quite alike in their cultural characters. Ashford has emphasized the production of a pale-greenish tint in old agar slant cultures of *Monilia psilosis*, but this same color appears in equal intensity in cultures of *Monilia albicans* of the same age. Castellani has described a few rare forms with yellowish or brownish pigment. Fischer and Brebeck described two forms from thrush, one of which liquefied gelatin (and formed ascospores, as mentioned above), the other not liquefying. But liquefying forms from thrush have not been observed by subsequent workers, though Plaut [36] describes a "softening," not true liquefaction, of beer-wort gelatin. It is probably on this basis that Castellani lists *Monilia albicans* as a gelatin liquefier.

Sugar Fermentations.—The fermentation of carbohydrates seems to be the only laboratory procedure by which the various Moniliae may be differentiated. The use of such reactions has been carried to an extreme by Castellani,[14] who recognizes a multiplicity of species as indicated by the accompanying table.

In this table only the production of gas from the sugars is recorded. But many strains will produce acid without gas from galactose and sucrose, as was noted by Fineman. It is interesting that sucrose is fermented without gas production, whereas dextrose and levulose are broken down to form both acid and carbon dioxide. It would seem that these organisms attack sucrose directly, without first converting it to the monosaccharides. Small amounts of alcohol are produced from the monosaccharides.

Castellani's conception of the plurality of species has been rather strongly criticized by Ashford, by Manson-Bahr, and by Mackie and Chitre on the ground that the reactions are not constant, the ability to ferment certain sugars being lost or acquired after continued cultivation. To this criticism Castellani has

BIOCHEMICAL CHARACTERS OF CERTAIN MONILIAS *

	Dextrose	Levulose	Maltose	Galactose	Saccharose	Lactose	Mannitol	Dulcitol	Dextrin	Raffinose	Arabinose	Adonitol	Inulin	Sorbitol	Starch	Glycerin	Inositol	Salicin	Amygdalin	Isodulcitol	Erythrite	Gelatin	Serum	Litmus Milk	Color of Growth on Dextrose Agar
Monilia zeylanica Castellani	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	Yellowish
Monilia zeylanoides Castellani	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White†
Monilia (Cryptococcus) macro glossiae Castellani	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White‡
Monilia balcanica Castellani	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia parabalcanica Castellani	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia krusei Castellani	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia parakrusei Castellani	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia (Cryptococcus) castellanii Re	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Yellowish or reddish brownish
Monilia pinoyi Castellani	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia nabarroi Castellani	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia metalondinensis Castellani	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia pseudolondinensis Castellani	G	G	G	G	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia alba Castellani	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia pseudolondinoides Castellani	G	G	G	G	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia africana Macfie, 1921	G	G	G	0	G	0	0	0	G	G	0	—	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia albicans Robin, emendavit Castellani and Chalmers	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	White
Monilia tropicalis Castellani	G	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia metatropicalis Castellani	G	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia rhoi Castellani	G	G	0	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia guilliermondi Castellani	G	G	0	0	G	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia macedoniensis Castellani	G	G	0	G	G	0	0	0	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	C	White
Monilia macedoniensoides Castellani	G	G	0	G	G	0	0	0	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	White
Monilia chalmersi Castellani	G	G	0	G	G	0	0	0	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	Dark brown
Monilia bronchialis Castellani	G	G	G	0	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia pseudotropicalis I Castellani	G	G	0	G	G	G	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia pseudotropicalis II	G	G	0	G	G	G	0	0	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	White
Monilia pseudotropicalis III	G	G	0	G	G	G	0	0	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	C	White
Monilia pseudotropicalis IV	G	G	0	G	G	G	0	0	0	G	0	0	G	0	0	0	0	0	0	0	0	0	0	0	White

* G indicates production of gas; C, production of clot; 0, negative result; —, gas absent, no clotting.
† Does not produce any discoloration of lead agar.
‡ Produces black discoloration of lead agar.

replied, not without reason, that it is the sugars which a strain will ferment when first isolated, not those which it can be educated to ferment, that are of diagnostic importance. Nevertheless, he has sufficient faith in the constancy of the fermentation reactions of the Moniliae to propose using them as a means of identifying unknown sugars.

In the author's opinion a more serious criticism of the differentiation of species by sugar fermentations is the failure of these characters to correlate with any other features. The reactions with carbohydrates are important in the colon-typhoid group of bacteria because they enable us to differentiate pathogenic species from harmless ones, and different diseases from each other. But Castellani himself points out that Moniliae with the same fermentation reactions may be obtained from diverse types of lesions, or may be quite non-virulent; conversely many different "species" may be isolated from the same sort of lesion, as in thrush. And since there are no morphological characters or other cultural dif-

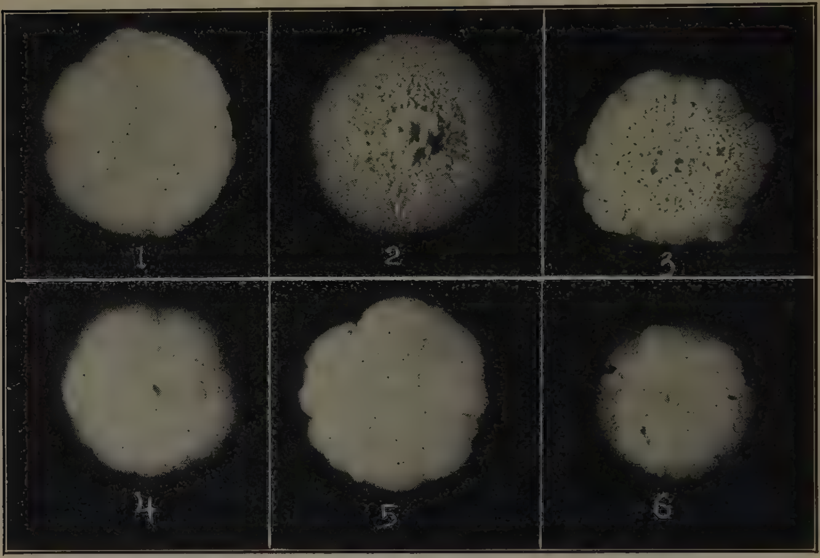

FIG. 81.—Giant colonies of pathogenic Moniliae. 1, 2, from cases of oral thrush. 3, 4, from cases of interdigital erosion. 5, 6, sprue strains obtained from Dr. Ashford.

ferences that correlate with the sugar fermentations, it would seem futile to multiply species on this basis.

The question of unity or plurality of species of Moniliae of this type can by no means be considered closed. Moniliae are associated with such a wide variety of disease processes that one cannot help but feel that these are not all caused by the same organism, even though no tangible means of differentiation has been brought forth. The whole problem needs very intensive investigation. But in the present state of our knowledge it would seem that we can justly recognize but two species, the saprophytic *Monilia candida* and the pathogenic *Monilia albicans*.

Monilia candida.—*Monilia candida* was first described by Hansen. It was isolated from manure and from fruits, but has since been obtained from a variety of sources. Anderson [1] found it in human feces. It differs from the pathogenic species in the formation of a pellicle on liquid media, and lack of virulence for laboratory animals. It produces a much more vigorous alcoholic fermentation than the pathogenic species. Another saprophytic form described by Fischer and Brebeck [21] as frequently labeled *Monilia candida* differs markedly from Hansen's description in its failure to form a pellicle and to grow at incubator temperature. Both of these forms ferment dextrose, levulose, maltose and sucrose vigorously. The fermentation of sucrose is direct, i.e., without a preliminary inversion.

The Moniliases.—The Moniliae are primarily parasites of the mucous membranes. Thus thrush occurs most frequently in the mouth, and not uncommonly in the vagina (in pregnancy). It may also extend through the gastro-intestinal tract, and more rarely involve the respiratory passages. The Moniliae associated with sprue are also found mainly in the mouth and intestines. There have been reported some primary infections of the bronchi and lungs not associated with thrush. In some cases of thrush, the infection may involve the skin, producing eczema-like lesions of the moister parts, and also involving the finger-nails. But there have also been recorded a considerable number of cases of Monilia infection of the skin, particularly of the folds between the fingers and about the finger-nails, not associated with thrush. Finally, a skin disease, psoriasis, has been considered as caused by a species of Monilia by Fleisher and Wachowiak.[24]

In a few cases of thrush, invasion of the blood stream with the production of metastatic abscesses has occurred. Fleisher and Wachowiak [22] record obtaining Moniliae in blood cultures from a case of sprue, and two other cases of diarrhoea not associated with sprue. A case of subcutaneous abscess caused by a Monilia has recently been reported.[15]

Thus, Moniliae may be associated with a variety of disease processes. Whether they are the primary etiologic agents in all of these cases is as yet uncertain. It should be kept in mind that organisms of this group may be found in a certain proportion of healthy individuals, in the mouth, throat or intestines. Thus Tanner, Lampert and Lampert [41] found yeast-like fungi (apparently mostly *Monilia albicans*), in 10 per cent of normal throats. Anderson [1] found yeasts or yeast-like organisms from the feces of 47 per cent of the individuals examined; these were, however, for the most part "wild yeasts" not like the Moniliae. But Mackie and Chitre [29] in Bombay found *Monilia psilosis* present in the feces of cases of diarrhoea and in normal individuals as frequently (40 per cent) as in sprue cases.

Thrush.—Thrush is one of the commonest of the mycoses of man. It is not so common or so serious nowadays as it has been in the past. It is most frequently a disease of nursing infants, particularly when they are undernourished or where there is a lack of cleanliness in their care. In older medical literature one reads of great epidemics of thrush in foundling asylums, with a relatively high mortality. We seldom see cases in such frequency or severity now. Thrush also occurs in adults, generally as a terminal event in such wasting diseases as typhoid fever, tuberculosis, or cancer, especially in patients who have been comatose or nearly so for some time, where the dryness of the mouth seems to favor the infection. It also occurs frequently as a mild infection of the vagina in pregnant women.

The majority of the cases are mild, and the infection remains localized to the affected mucous membrane, giving rise to no symptoms save local irritation. In such cases the disease appears as soft whitish patches on the mucous membrane of the mouth, on the tonsils, cheeks or gums, occasionally on the tongue. These patches, composed mainly of the fungus growth, can generally be easily removed, leaving a slightly abraded surface. The membrane may be mistaken for a diphtheritic membrane, and judging by the frequency with which this organism is found in throat cultures sent to diagnostic laboratories for examination for diphtheria bacilli, the disease must frequently be confused with diphtheria by practitioners. Fineman obtained some of her strains of the thrush parasite from such cultures, and Tanner and Dack [40] investigated 22 strains of yeast-like organisms obtained from cases of sore throat which were for the most part *Monilia albicans*.

In some cases the disease may become chronic and spread to other mucous membranes and the skin. There have been reported a small number of such cases in children, in which the organisms were present in the mouth over a period of some years, with occasional repeated infections, accompanied by eczematoid lesions of the skin, especially in the moist parts, between the thighs, in the bends of the elbows, and particularly between the fingers and toes. In several such cases observed by the author, loss of hair was a striking feature, although actual infection of the scalp could not be demonstrated. In these cases the organism could be regularly isolated from the feces in considerable numbers. These cases usually terminate fatally. The generalized cutaneous eruptions suggest that the condition is similar to that designated "trichophytide" in the ringworms, either an allergic manifestation or a blood-borne distribution in which for some reason the organism localizes in the skin only. It is, however, quite possible that the organisms pass through the intestinal tract and are carried from the mouth or the anus to the skin surfaces.

In a small number of cases (seven collected by Plaut), generalized infection by way of the bloodstream with metastatic abscesses in internal organs have occurred.

The diagnosis of thrush is established by microscopic examination of the membrane. This is best done by the use of unstained wet preparations. One finds a tangled mass of branched filaments of segmented mycelium, and scattered irregularly through them a number of yeast cells showing budding, together with leucocytes and desquamated epithelial cells. The organism is easily isolated in pure culture by the use of dextrose tartaric acid agar plates. It is to be differentiated from yeasts which occur not infrequently in the throat, by the gelatine stab cultures described above.

Freshly isolated strains are usually pathogenic for rabbits, producing localized abscesses, occasionally generalized infections. Intravenous inoculations produce a generalized infection with miliary abscesses especially in the cortex of the kidneys, that resemble closely the lesions produced by *Aspergillus fumigatus.* In these lesions both mycelium and yeast cells may be found. Mice are very susceptible.

Sprue.—Sprue is a disease of the tropics, occurring apparently in all parts of the tropical zone, and seen not infrequently in temperate climates in returned missionaries and others. It is a slowly developing, chronic, progressive disease, characterized by an inflammation of the mouth, particularly the tongue, leading gradually to an atrophy of its mucous membrane; a frothy diarrhoea accompanied by a failure to digest fats, by flatulence and other gastro-intestinal symptoms; and a severe anemia of a type approaching pernicious anemia.

Although Moniliae had been isolated from cases of sprue by several preceding workers, attention to this group of organisms as possible causative agents is largely due to the investigations of Ashford,[2–6] who succeeded in obtaining a Monilia regularly from scrapings of the tongue and from diarrhoeal stools of the majority of cases investigated. He claimed this organism to be a species distinct from *Monilia albicans* and named it *Monilia psilosis* (it has been renamed *Parasaccharomyces ashfordi* by Anderson).

Concerning the relation of this organism to sprue there are three views held by different workers: That it is the causative agent; that it produces a secondary infection, the primary cause of the disease being a dietetic or glandular deficiency; and that it has nothing to do with the disease, being a normal mouth and intestinal parasite.

The original view of Ashford that this organism is the sole cause of sprue has had little acceptance, and has been finally abandoned by Ashford himself, though he still believes that the Monilia is responsible for a large part of the clinical picture. The majority of investigators seem to lean toward the second theory, that the organism finds a foothold in individuals weakened by the disease, and is perhaps responsible for some of the inflammatory reactions of the mucous membranes. The third viewpoint is that of Mackie and Chitre,[29] who found *Monilia psilosis* as frequently in normals as in sprue cases.

Ashford has insisted that his organism is quite different from *Monilia albicans*, mainly on the grounds of morphology. But such morphologic differentiation cannot be made by other observers. Of the sugar fermentations, he states that dextrose, levulose, and maltose are regularly fermented, galactose and sucrose sometimes. The fermentation of maltose is insisted upon as a means of differentiation from "wild yeasts" in the feces. *Monilia albicans* also ferments dextrose, levulose, and maltose regularly, but does not form gas from galactose and sucrose. It would appear from examination of tables of fermentation reactions published by several authors, that strains of Monilia from sprue produce gas from sucrose in the majority of cases. A differentiation from *M. albicans* might be established on this basis. Hines [25] discusses the difficulty of differentiating this species, and concludes that morphologic and biochemical reactions are not sufficiently distinctive to make a differentiation possible.

Freshly isolated strains of *Monilia psilosis* inoculated into animals appear to produce the same types of reactions as *Monilia albicans*, namely, localized abscesses, or a generalized infection with miliary abscesses in internal viscera. Ashford claims to have produced, with strains whose virulence was increased by animal passage, stomatitis and diarrhoea in feeding experiments. Wood [50] also claims to have produced a haemolytic anemia in guinea pigs by feeding cultures of this organism; a filtrate of this organism when injected intravenously into rabbits, also seemed to possess haemolytic properties. On the other hand, Weiss and Landron [46] could not demonstrate any haemotoxine in vitro.

The evidence from animal experiments is far from being conclusive that this organism is responsible for the disease. The evidence available then is limited to demonstrating its constant presence in the disease and absence in normal individuals. On this point there is a great deal of difference of opinion, as indicated above. Thus Ashford found the organism in 953 of 1093 cases of clinical sprue, and in only 10 of 655 cases known not to have sprue. Although Anderson [1] found yeast-like fungi in nearly half of the cases not sprue examined by him, none of the isolated strains could be identified as *Monilia psilosis*. On the other hand, Mackie and Chitre found the organism in only 40 per cent of the cases of sprue examined by them, and in as large a proportion of cases of non-sprue.

Dermatomoniliases.—In addition to the cutaneous lesions observed in cases of thrush, Moniliae have been isolated from various skin affections not associated with lesions of the mucous membranes. These have been, for the most part, conditions similar to those produced by *Epidermophyton cruris*, namely eczema-like lesions of the moist skin areas. They have been noted especially frequently in the folds between the fingers, especially in washerwomen and housewives. The disease is known as "erosio interdigitalis." An infection about the finger-nails occurs in workers in fruit canneries in the Northwest, which according to Kingery and Thienes [26] is caused by Moniliae. These have been isolated from pears by Thienes [42] and found identical with the species isolated from the workers. No characters are given by which they can be distinguished from *Monilia albicans*.

Recently Wachowiak [44] and his associates have suggested a possible relationship of Moniliae in the etiology of psoriasis. This is based upon finding such organisms in the feces in a much higher proportion (86 per cent) of cases than in normal individuals; upon obtaining positive blood cultures in 17.5 per cent of the psoriasis cases; and upon isolation of the organism from the skin lesions in 36.5 per cent of the cases. They could not reproduce the disease by inoculating either animals or people.

Bronchomoniliases.—Although it has long been known that in occasional cases the thrush parasite may invade the respiratory tract, within recent years there has been recognized a distinct type of Monilia infection of the lungs known as bronchomoniliasis. These cases were first observed by Castellani [12, 13] in Ceylon, and the majority have been reported from tropical countries, though not a few have been observed in England,[27] America and Europe.

Castellani recognizes two types of the disease. One is a mild form in which a chronic cough with scanty mucopurulent sputum is the main symptom. This type he refers to as "tea-taster's cough," since it was observed in tea tasters. A similar condition occurs in the workers in tea factories. Moniliae may be constantly found in the tea. The other type is more severe, with fever, and resembles tuberculosis in its course. It may prove fatal. Various species of Moniliae as recognized by Castellani have been isolated, but *M. tropicalis* has been most frequently obtained.

Moniliae may be found very frequently in samples of sputum in cases in which the primary cause of the disease is clearly some other organism, particularly tuberculosis. Since such Moniliae may be found so frequently in the normal mouth or throat, it follows that the mere presence of Moniliae in sputum is not sufficient evidence to establish a diagnosis of bronchomoniliasis. This has been strongly emphasized by Castellani, who recommends that precautions should be taken to prevent contamination of the sputum from an outside source, that the throat should be cleansed by gargling before collecting the sputum, and especially that the virulence of the strain must be tested by animal inoculation. Strains from bronchomoniliasis inoculated into the lungs of rabbits give rise to tubercle-like nodules in two or three weeks.

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CHAPTER VIII

MORPHOLOGY AND CLASSIFICATION OF THE YEASTS

YEASTS are fungi that permanently maintain a unicellular growth form, not developing mycelium. Many fungi may temporarily assume such a form. Thus various Mucors give rise to budding single cells in semi-anaerobic cultures; in artificial cultures of the smut organisms only yeast forms appear, though mycelium is formed in the host plant; in the organisms of sporotrichosis, blastomycosis and coccidioidal granuloma, only single cells without mycelium appear in the tissues, but mycelium develops in artificial cultures; finally, with the Moniliae which have been considered in the preceding chapter, only yeast-like cells appear in artificial cultures if optimum growth conditions are maintained, but mycelium develops under unfavorable (e.g., semi-anaerobic) conditions. The discovery of these dimorphic types has led to a general assumption that the true yeasts have developed from such more complex forms, having permanently lost the ability to form mycelium. This assumption has received some experimental support in an observation by Fuchs,[6] who grew *Aspergillus oryzae* under semi-anaerobic conditions for some weeks, and noted a development of yeast-like cells from the submerged conidia, accompanied by a slight alcoholic fermentation. Subcultures of these yeast cells did not, however, return to the mold-form, but grew permanently as yeasts, even in aerobic cultures, and moreover produced endogenous spores of the hat-shaped form characteristic of the yeast, *Willia anomala.* If this observation can be confirmed, and it can be shown definitely that the author was not dealing with a contamination of his cultures (by Willia), it will have an important bearing upon the classification of the yeasts.

In the past the yeasts have been looked upon as a fairly homogeneous group, divided into two subdivisions: those forming endogenous spores, and those forming no spores. The spores have been looked upon as ascospores, particularly since the discovery of rudimentary sexual processes in some species, and therefore the two groups have been considered as subdivisions of the Ascomycetes and Fungi Imperfecti respectively. But very recently there have been discovered some rather peculiar modes of growth and reproduction in two new types of yeasts that indicate that the yeasts in general may not be so homogeneous in their origin as we have previously supposed. These new types reproduce by the formation of exogenous spores that have been interpreted as basidiospores and as conidia respectively.

If we exclude these two unusual genera, however, the remaining yeasts can be arranged fairly satisfactorily into two main divisions or families: the Saccharomycetaceae forming endogenous spores, and the Torulaceae forming no spores. The endogenous spores are considered to be ascospores, because they are in some cases typically eight in number and in some cases result from a conjugation of two cells. The yeasts are, therefore, classified with the Ascomycetes. This is further justified by the finding of transitional forms between the one-celled yeasts and the more complex filamentous Ascomycetes, i.e., forms which may be unicellular under certain conditions and multicellular under others. Such transitional forms are included in the genus Endomyces.

This genus belongs to the family of Endomycetaceae in the Exoascus group of the Ascomycetes, which group is characterized by the formation of naked asci, i.e., asci without any protective or supporting tissue. It forms spherical cells at the tips of the filaments of mycelium, much like conidia. But in liquid media these continue to grow and form new spherical cells by budding. In addition it forms ascospores by the conjugation of two neighboring cells in a filament of mycelium. In one species the ascospores have a very peculiar form similar to that of a derby hat (*Endomyces fibuliger*). Ascospores of this same peculiar form occur in a true yeast, *Willia anomala*. Thus it is clear that there is a close relationship between molds of the type of Endomyces and yeasts of the type of Willia. It is generally considered that yeasts have developed from molds by losing the power to form mycelium.

The genus Monilia may similarly be considered as transitional between the non-spore-forming yeasts and the molds of the Fungi Imperfecti. It may produce either mycelium or exist as round budding cells, like Endomyces, but lacks the ascospores. It has probably developed from perfect forms like Endomyces, and like other imperfect fungi, may be merely imperfectly known. With regards to the non-spore-forming yeasts, two possibilities present themselves: They may have arisen from spore-forming yeasts, having lost the power of forming spores, or from transitional fungi such as Monilia, having lost the power of forming mycelium.

The relationships of the two groups of yeasts and of transitional fungi are shown diagrammatically in Fig. 82.

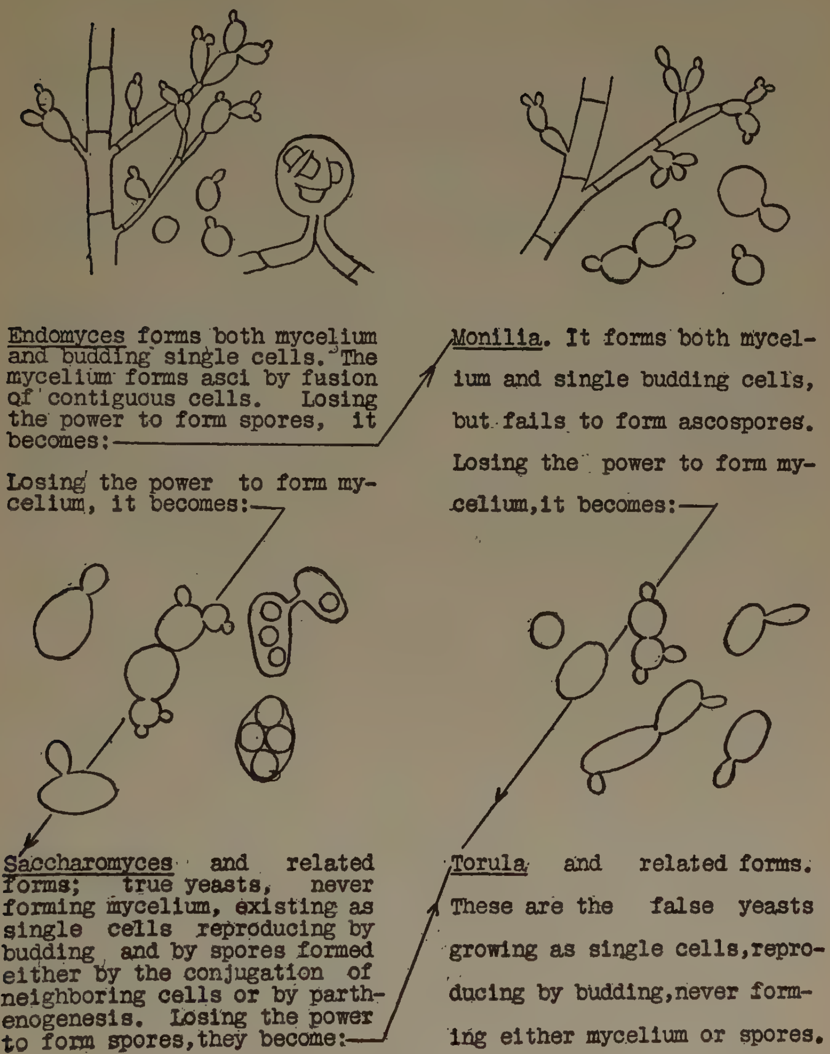
Cytology of Yeasts.—The structure of the yeast cell has been studied in some detail. It consists of a protoplast contained in a cell wall. The cell contains a single nucleus and numerous granules and vacuoles representing various kinds of reserve foodstuffs. When young, i.e., when the culture is actively growing, the cell wall is thin and the protoplasm is fairly free of these reserve substances. After growth ceases, the cells develop thicker walls and become filled with granules and vacuoles. Occasional cells in old cultures develop extraordinarily thick walls and become distended with a large amount of reserve material. These are referred to in German literature as "dauerzellen" and are, functionally at least, identical with the chlamydospores of mycelial fungi.

Nucleus.—The existence of a nucleus in yeasts, doubted for many years, was first established by Moeller. Numerous later researchers have attempted to establish further details of its structure and behavior in division, but it is usually so small that little definite information is obtainable. It appears as an almost homogeneous body near but not quite at the center of the cell, round, or, due to compression by a neighboring vacuole, frequently kidney-shaped (*n*, Fig. 83). It can only rarely be seen in unstained cells. It can certainly be demonstrated only in preparations carefully fixed and stained either with iron-haemotoxyline or one of the eosine-methylene-blue stains. In some preparations the nucleus appears somewhat irregular in form, and it is claimed that it possesses a power of ameboid movement through the cytoplasm. After a bud has started forming, the nucleus apparently moves to that side of the cell and sends a long prolongation into the bud, which prolongation becomes swollen at the tip. This swollen portion then becomes separated from the parent nucleus by first an attenuation and finally a rupture of the connecting strand of nuclear material. Mitotic division, claimed to have been observed by some authors, probably does not occur. Guilliermond [9] states

SYSTEMATIC RELATIONSHIPS OF THE YEASTS

ASCOMYCETES

FUNGI IMPERFECTI



Endomyces forms both mycelium and budding single cells. The mycelium forms asci by fusion of contiguous cells. Losing the power to form spores, it becomes:

Losing the power to form mycelium, it becomes:

Saccharomyces and related forms; true yeasts, never forming mycelium, existing as single cells reproducing by budding and by spores formed either by the conjugation of neighboring cells or by parthenogenesis. Losing the power to form spores, they become:

Monilia. It forms both mycelium and single budding cells, but fails to form ascospores. Losing the power to form mycelium, it becomes:

Torula and related forms. These are the false yeasts growing as single cells, reproducing by budding, never forming either mycelium or spores.

Fig. 82.

that a nucleolus is present, but Kohl[14] claims that this is an "albuminous crystalloid."

The remaining structures in the protoplast are all reserve food-stuffs and may be discussed under three heads: Fat, carbohydrates, and proteins.

Fat appears at times in old cultures as highly refractile vacuoles. At first these are usually numerous and small, but as the cell grows older they tend to coalesce and form one large vacuole which may nearly fill the cell. Such large fat vacuoles are very characteristic of certain species of yeasts. Their fatty nature may be readily demonstrated by staining with Sudan III.

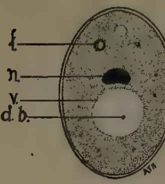


FIG. 83.—Diagram showing the structure of a yeast cell. *n*, nucleus. *v*, vacuole. *d.b.*, "dancing body." *e*, fat vacuole.

Carbohydrate, as in the more complex fungi, is stored as glycogen and can be demonstrated by suspending some of the culture in Gram's iodine solution and examining with the high-power lens under a coverglass. The glycogen granules stain a deep reddish brown, the rest of the cell a pale yellowish brown.

Protein occurs in the cytoplasm in the form of fine granules which have been studied by Kohl[14] and by him designated albuminous granules. They may be demonstrated by overstaining with the basic dyes and then differentiated by some decolorizing agent. For this purpose Gram's stain serves admirably.

Gram's Stain.—In connection with these albuminous granules some observations by R. and W. Albert[1] and by the present author[12] are of interest, since they perhaps throw some light on the nature of Gram's stain as well as of the granules in question. If fresh preparations of young yeast cells are stained by Gram's method, all of the cells stain a uniform deep blue-black. The Alberts showed that, if yeast cells were killed by a procedure which did not destroy their enzymes (treatment with acetone) and were then suspended in water, they would undergo autolysis. If now one makes preparations of these autolyzing cells and stains by Gram's method, it will be found that, as autolysis proceeds, part of the protoplasm fails to retain the Gram's stain, and large numbers of Gram-positive granules appear in the Gram-negative matrix. As autolysis proceeds, these granules become fewer in number and finally disappear. The disappearance of the granules is accompanied by increasing amounts of protein in the water, i.e., there is a correlation between the Gram-staining of the granules and their resistance to autolysis.

If now one prepares a series of slides of fresh, non-autolyzed yeasts, stains by Gram's method, and decolorizes with different strengths of acid alcohol instead of the plain alcohol ordinarily used, an exactly similar series of preparations is obtained. That is, a slide of fresh yeast decolorized with say 1 per cent acid alcohol will appear like a slide of yeast which has autolyzed say 1 hour; decolorized with 5 per cent acid alcohol it will appear like a slide of yeast which has autolyzed two hours; and so on. Thus one may demonstrate in the protoplasm of the freshly fixed yeast cell a whole series of fine protein granules which vary in their tenacity of Gram's stain, and this variation is correlated with their resistance to autolysis after the cell has died. These studies also indicate that the Gram-staining property is not resident in the membrane, as has been claimed, but in proteins contained in the protoplasm.

Volutin.—Another very prominent feature of the yeast cell is the presence of material known as volutin or metachromatic material. It occurs in all of the higher fungi and in bacteria as well, but is especially prominent in the yeasts. Practically every cell contains at least one vacuole filled with this material situated near the nucleus (*v*, Fig. 83). They are less refractile than the fat vacuoles. Generally the vacuoles contain a small granule which exhibits an active Brownian movement, frequently referred to as the "dancing body" (*db*, Fig. 83). This Brownian movement indicates that the vacuole contains fluid of not very high viscosity. The granule within the vacuole may sometimes suddenly disappear, and after a time as suddenly reappear. This fact, together with the fact that both the fluid of the vacuole and the granule behave the same toward certain vital stains, indicates that they are both of the same nature. The granules represent some of the material of the vacuole which has temporarily coagulated. In older cells additional smaller vacuoles are found, and sometimes granules of the same material not within a vacuole.

In slides fixed by the usual procedures, the material in the vacuoles coagulates and becomes shrunken and distorted, appearing as irregular masses in the cytoplasm. They take the basic dyes intensely and in general stain as does the chromatin, but may be differentiated from the nucleus by various methods. They are most beautifully demonstrated, however, by vital staining with neutral red. Some of the cells suspended in water are placed under a coverglass and a drop of 1 per cent aqueous solution of neutral red is applied to one edge of the coverslip. As this seeps under, a solution of graded concentration is formed, and in some part of the field the right concentration will be found. Dead cells stain a uniform red with this dye, but in living cells only the volutin vacuoles and granules will stain, the former a light pink, the latter a deep red.

It is generally agreed that the volutin of yeasts and bacteria is a reserve substance, either free nucleic acid or a nucleic acid compound different from the nucleoproteins of the nucleus. Schumacher[18] claims to have demonstrated that it is free nucleic acid by microchemical reactions. The amount of volutin developed in the cells appears to be proportional to the amount of nucleic acid which can be extracted, and also to a certain extent to the phosphorus available in the medium. The volutin may be absent in very young cultures, accumulates in large amounts in old cultures, particularly in the "dauerzellen," and is used up or disappears in spore formation. There has been some attempt to correlate the amount of volutin in the cells with degree of fermentative activity, but this is apparently not true. Volutin may appear in large quantities in yeasts with no fermenting power at all.

Multiplication.—The vegetative multiplication of the yeasts is accomplished by budding, by fission, or by a combination of these two processes. The last two methods are confined to a few species. In simple budding the cell wall apparently softens at one point and the protoplasm bulges it out. The bud generally reproduces the form of the parent cell. When the bud is mature, it is separated from the parent cell by constriction of its base. When rapidly growing, several buds may form simultaneously from different parts of the cell, and these may give rise to new buds before they have become detached from the parent cells, so that a small cluster of cells is formed. Reproduction by fission is the same process as occurs in bacteria. After elongating to a sufficient length, a cross-wall is laid down, and this then splits in the middle, giving rise to two cells of equal size. In the combination process buds develop as above, but, instead of being separated from the parent cell by constriction, a cross-wall develops in the pedicel of the bud.

Spores.—In addition to these methods of vegetative multiplication, yeasts may reproduce by intracellular spore formation. These spores are somewhat like those of bacteria in that they are more resistant to heat and drying than are the vegetative cells, but unlike bacterial spores in that they are generally multiple and therefore truly reproductive; and that they are not formed readily as soon as the culture has reached a certain age, but only under exceptional circumstances. To demonstrate yeast spores it is necessary to subject a vigorously growing culture rather suddenly to unfavorable conditions, as was described in Chapter II.

In the majority of the yeasts the spores are formed by a repeated division of the nucleus, generally twice, forming four spore nuclei. About these nuclei the protoplasm becomes condensed, there is formed a spore wall and the spores are mature. In this process each cell is independent of the others, there is no sexuality, and the spore formation is said to be parthenogenic.

Sexuality.—In some of the yeasts, however, spore formation is preceded by a sexual fusion of two cells, the spores developing after the division of the fused nuclei.

The sexuality of the yeasts may be manifested in various ways, most clearly in isogamic conjugation. Neighboring cells of equal size send out small tubular processes towards each other. These meet and fuse. Their nuclei meet and unite, the fusion nucleus undergoes division the proper number of times, and the spores proceed to mature as above (Fig. 12).

In other cases spores may develop following the formation of a bud, the nucleus of the bud returning to the parent cell and fusing with the parent nucleus. This is considered heterogamic conjugation. In still other cases spores may be formed without any conjugation, but when the spores germinate they fuse two by two, usually within the ascus. In some yeasts conjugation never occurs, though when spores are formed, narrow tube-like processes are formed as in those cases where conjugation does occur, but these never unite with the processes from other cells.

In the yeasts, then, we see all stages in a retrograde evolution from an organism reproducing by a sexual process, through the loss of sexuality and finally the loss of the power to form spores at all. These observations, largely the work of Guilliermond,[9] have thrown considerable light on the origin and evolution of the yeasts, and furnish a basis for classification.

Germination of Spores.—Yeast spores germinate when transferred to a favorable medium, generally by simple imbibition of water and a gradual swelling to assume the form of the vegetative yeast cell. In one group the spore has a double membrane, and on germination the outer membrane is ruptured and cast off, the inner membrane serving as the cell wall of the new vegetative cell. In some cases the ancestry of the yeasts in the molds is still further indicated by the spores sending out a short filament of mycelium (promycelium) on germination, from which vegetative yeast cells are formed by lateral budding.

Yeasts with Endospores: Saccharomycetaceae.—The key to the genera of spore-forming yeasts presented below is based upon the arrangement and descriptions of Guilliermond,[9] with the addition of the genus Zygosaccharomycodes recently described by Nishiwaki.[16]

A. Reproducing by fission. *SCHIZOSACCHAROMYCES.*

B. Reproducing by budding or by a process intermediary between budding and fission.

- 1. Spores formed by isogamic conjugation.
  - a. Multiplication by budding. *ZYGOSACCHAROMYCES.*
  - b. Multiplication by a process intermediary between budding and fission. *ZYGOSACCHAROMYCODES.*
- 2. Spores formed by heterogamic conjugation.
  - a. Spores with a single verrucose membrane. *DEBAROMYCES.*
  - b. The entire cell becomes the spore. *NADSONIA.*
- 3. Spores formed without conjugation, but fusion tubes are formed.
  - a. Spores with a projecting collar *SCHWANNIOMYCES.*
  - b. The entire cell becomes the spore. *TORULOSPORA.*
- 4. Spores formed without any traces of sexuality.
  - a. Growing at the bottom of liquid cultures, forming no pellicle at all, or only after growth has ceased.
    - aa. Multiplying by a process intermediary between budding and fission.
      - I. Spores with a single membrane. *SACCHAROMYCODES.*
      - II. Spores with a double membrane. *SACCHAROMYCOPSIS.*
    - bb. Multiplication by true budding.
      - I. Cells not lemon-shaped. *SACCHAROMYCES.*
      - II. Cells lemon-shaped. *HANSENIA.*
  - b. Growing in liquid media as a pellicle from the beginning. Pellicle is dry and entraps gas bubbles.

aa. Spores round or hemispherical. *PICHIA.*
bb. Spores lemon-shaped or hat-shaped. *WILLIA.*

C. Yeasts not well known.
1. Budding yeasts with a single needle-shaped spore. *MONOSPORA.*
2. Budding yeasts with four spindle-shaped motile spores. *NEMATOSPORA.*
3. Budding yeasts with eight spindle-shaped spores. *COCCIDIASCUS.*

Schizosaccharomyces.—The genus Schizosaccharomyces seems to be mainly a tropical group of yeasts. Species have been isolated from various tropical fruits, from African beer made from millet, from Jamaican molasses, and from various insects. Most species form from four to eight spores by isogamic conjugation. One species without spores has been described.

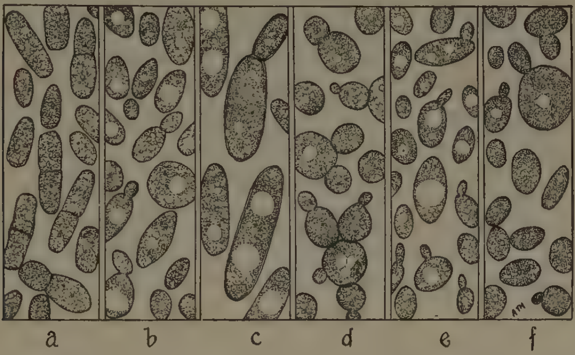


Fig. 84.—Vegetative cells of some yeasts. a. *Schizosaccharomyces pombé.* b. *Zygosaccharomyces bailii.* c. *Saccharomycopsis guttulatus.* d. *Saccharomyces cerevisiae.* e. *Saccharomyces ellipsoideus.* f. *Saccharomyces fragilis.*

Zygosaccharomyces.—In Zygosaccharomyces are grouped together a number of yeasts reproducing by budding in which the spores are formed by conjugation. The number of spores varies, but is usually four. In most cases the conjugating cells are of equal size, but in some conjugation is heterogamic, while in many cases all gradations may be found. A considerable number of species has been isolated from a variety of sources, as ginger beer, Soya sauce, from leaf mold, from soil, and from ketchup. Most of them produce a fermentation but are not of industrial importance save as a cause of spoilage. Lochhead and Heron found members of this genus to be responsible for the fermentation of honey.

Zygosaccharomycodes.—This genus has recently been created by Nishiwaki [16] to include a new yeast isolated by him from saké, which combines the isogamic conjugation characteristic of Zygosaccharomyces with the peculiar type of budding found in Saccharomycodes. It forms a dark sediment in saké and is responsible for an after-fermentation in that beverage.

Debaromyces.—The genus Debaromyces is characterized by the formation of a single spore which is covered with small prickle-like elevations. Spores may be formed by either isogamic or heterogamic conjugation, usually the latter. Species have been described (by Klöcker) from soil and from a Russian cheese. Recently Ota has identified several yeasts previously isolated from various human lesions as belonging to this genus.

Nadsonia.—The genus Nadsonia contains rather peculiar yeasts. The vegetative cells are large and frequently form a small nipple-like projection at one end. In old cultures many of the cells form two large buds, one at either end. One of these may be cast off. The nucleus of the other fuses with the nucleus of the parent cell, following which, according to Guilliermond,[9] a spore is formed. But apparently the entire cell becomes the "spore," which becomes filled with an enormous fat globule. The membrane of such a cell is greatly thickened, and after a time the outer lamella of this wall peels off irregularly, leaving a roughened or verrucose outer surface.

The growth of *Nadsonia fulvescens* in young cultures is white and very mucoid in character. The cells are very large. With the development of the fat globules in the "spores" the growth gradually becomes tan and then reddish brown in color. In many cultures white sectors appear in the old cultures, and these are found to contain non-sporogenous cells free from fat globules, which when subcultured yield a similar pure white growth.

Schwanniomyces and Torulospora.—In each of these genera the cells produce, under conditions which favor sporulation, slender tubular outgrowths as though they were about to undergo sexual fusion, but actual conjugation does not occur. In Schwanniomyces the cells then develop a single spore each, which shows a projecting collar with a prickly surface, as in Debaromyces. In Torulospora no true spore develops, but the entire cell becomes rounded and develops a large fat globule. Here the whole cell is looked upon as a spore, as in Nadsonia.

Saccharomycodes and Saccharomycopsis.—Two species of Saccharomycodes have been described, one from the sap of an oak tree, the other from hops. These yeasts form buds which, instead of becoming separated from the parent cell by constriction of the base, form a cross-wall at the base which then splits through the middle.

There is but one species of Saccharomycopsis, *Saccharomycopsis guttulatus.* This yeast is a constant intestinal parasite of rabbits, and is found occasionally also in the feces of guinea pigs. It is extraordinarily large. Budding occurs as in Saccharomycodes, but it is said to form spores with a double cell wall. These spores are said to be formed abundantly in rabbit feces. The author has been struck by the great similarity between the oöcysts of the Coccidium of rabbits and the published illustration of the asci of Saccharomycopsis and suggests that these may have been mistakenly identified. The author has been unable to find the sporulating forms of this yeast in rabbit feces. This yeast is not easily cultivated, but has been grown upon acidified glycerine gelatin.

Saccharomyces.—The genus Saccharomyces contains all of the yeasts of industrial importance. It is characterized by reproduction by budding, and by the formation of spores without any trace of a sexual process. *Saccharomyces cerevisiae* is the best known species, being the common brewing and baking yeast. It has been very intensively studied because it is of such practical importance, and is usually taken as the type of the yeasts for physiological studies. But there are many species of this genus, as well as differentiated strains or races of the cultivated species. The genus has been subdivided into groups as below, on the basis of sugar fermentations.

Group I ferments dextrose, maltose and sucrose, but not lactose.

Group II ferments dextrose and sucrose, not maltose or lactose.

Group III ferments dextrose and maltose, not sucrose or lactose.

Group IV ferments dextrose only.

Group V contains all species which ferment lactose.
Group VI contains species which do not ferment any of the sugars.

Industrial Yeasts.—The important industrial yeasts belong in the first group.

Saccharomyces cerevisiae forms rather large cells, either round or plump ovals in form. Spores vary from one to five in number, usually four. They are round and highly refractile. The optimum temperature for spore formation is 30° C.

Brewing yeasts are divided into two classes, known as top yeasts and bottom yeasts respectively. Top yeasts tend to remain attached to each other in clusters and to produce carbon dioxide vigorously, so that they are carried to the top of the fermenting liquid in a froth. Bottom yeasts grow and ferment in the depths of the liquid. In general they do not produce so much alcohol as top yeasts.

The introduction of pure cultures in the fermentation industries by Hansen naturally led to the selection of particular strains of yeasts chosen because they give rise to certain types of desirable fermentations. These have been given various "trade" names. Brewing yeasts are generally designated by names indicating the brewery from which they had their origin, as Carlsberg Bottom Yeast No. 1, etc. The Saaz, Logos and Frohberg yeasts are particularly well-known strains.

Yeasts used for bread making and compressed yeast manufacture are generally top strains of *Saccharomyces cerevisiae.*

Saccharomyces ellipsoideus is found naturally on grapes. It is also abundant in the soil of vineyards. The cells are elliptical in form. One to four spores are formed, the optimum temperature being 25° C. While it will generally outgrow other yeasts or molds present upon grapes if the conditions of fermentation are controlled, particular strains are also used as starters in wine making. They are named for the type of wine for which they are particularly adapted, as Tokay No. 1, etc.

Saccharomyces pastorianus is a frequent contaminant in brewing and may usually be obtained from commercial yeast cakes. It forms large sausage-shaped cells and a scum in old cultures. It gives rise to disagreeable flavors in beer.

The yeasts used to produce cider, Japanese saké, and various other fermented beverages, also fall in this first group. Of the remaining groups there are no very important species save those of the fifth, fermenting lactose.

Lactose Fermenting Yeasts.—There are in use in various parts of the world, particularly southeastern Europe, fermented beverages made from milk. Kefir and koumyss are the best known of these. The fermentation is a complex one, as pure cultures are not used, and there are various bacteria and usually several species of yeast present. Not all of the latter are capable of fermenting lactose, but depend upon bacteria for the preliminary conversion of the lactose to dextrose and galactose. These hexoses are then fermented to carbon dioxide and alcohol by the yeasts.

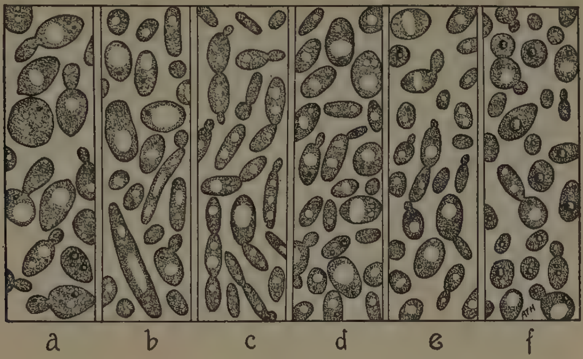


Fig. 85.—Vegetative cells of some yeasts. a. *Nadsonia fulvescens.* b. *Willia anomala.* c. *Pichia membranafaciens.* d. *Torula cremoris.* e. *Torula glutinis.* f. *Torula pulcherrima.*

There are, however, some yeasts which form lactase as well as zymase, and can carry on the alcoholic fermentation by themselves. Of these the best known is *Saccharomyces fragilis* (or *S. kefir*). The cells are oval to elliptical. From two to four spores are formed. It is a bottom yeast. Only a small amount of alcohol is formed, but an abundance of carbon dioxide. The resultant beverages are effervescent rather than intoxicating.

Hansenia.—The genus Hansenia, characterized by lemon-shaped cells, forms spores which are round, with a projecting ring or collar. There are two species: one regularly forming but one spore, the other two. They are found on fruits, and occasionally in wine, but are of no practical importance. Because they are so easily recognized, these yeasts were used by Hansen in studying the life cycles of yeasts in nature.

Pichia and Willia.—Pichia and Willia are readily recognized by the fact that they form dry, wrinkled pellicles on liquid media, which entrap gas bubbles during fermentation, and by the fact that in the fermentation of sugars they form esters (ethyl acetate, amyl acetate, etc.), which give very characteristic odors. An abundance of carbon dioxide is also formed but no alcohol. They are rather common forms, and of importance as contaminators in alcohol production, since they not only use up the sugar without yielding any alcohol, but also consume the alcohol which has been formed by other yeasts.

The genus Pichia is characterized by the formation of cells which are sausage-shaped, and which tend to remain attached to each other in a characteristic arrangement, forming a network on the surface of the medium. They form spores which are round, hemispherical or somewhat angular in form, according to the species. Each spore contains a very characteristic small, highly refractile fat globule, which is diagnostic of this genus.

The genus Willia is characterized by the formation of spores of peculiar form. There are two well-known species. In *Willia anomala* the spores are hemispherical and have a projecting rim like a derby hat. In recently isolated cultures these spores are formed very readily. This species produces ethyl acetate in fermentation, which gives the culture a very characteristic fruity odor. As was mentioned above, this yeast is very closely related to the mold *Endomyces fibuliger*, which also forms hat-shaped spores and ethyl acetate. In *Willia saturna* the spores are somewhat disc-shaped, possess an equatorial collar or ring, and contain a fat globule.

Some Unusual Yeasts.—The genus Monospora was created to include a single species found by Metchnikoff parasitic in the microscopic arthropod Daphnia. It forms a single needle-shaped spore which penetrates the digestive tract and enters the body cavity. It was with this organism that Metchnikoff made some of his first observations of phagocytosis.

Nematospora contains two species of yeasts, or yeast-like organisms which form oval to elliptical cells reproducing by budding, and eight spindle-shaped ascospores. The latter possess each a flagellum by which they are motile for a time. This disappears when they germinate. One species was found on hazel nuts, the other on tomatoes.

Coccidiascus is parasitic on the fruit fly Drosophila. The vegetative cells are round and reproduce by budding. Eight spindle-shaped or banana-shaped spores are formed.

Yeasts without Spores: Torulaceae.—Hansen first distinguished between spore-forming and non-spore-forming yeasts, finding the former to be desirable agents of alcoholic fermentation, the latter to cause frequent failures or undesirable fermentations in brewing. To the latter he gave the name Torula; they have frequently been designated "wild" yeasts to distinguish them from the spore-forming cultivated yeasts. Not much attention has been given them until recent years, although such yeasts are found in routine work much more frequently than are sporogenous species. A number of species have been described, but usually have been insufficiently described so that reidentification is frequently impossible.

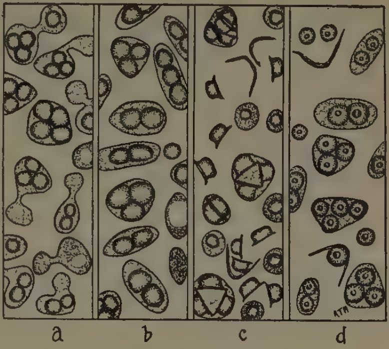


FIG. 86.—Asci and ascospores of some yeasts. a. *Zygosaccharomyces* species from soil. b. *Saccharomyces ellipsoideus.* c. *Willia anomala.* d. *Pichia* species from soil.

Will's Classification.—In 1916 Will [19] offered the first attempt at systematization of the non-sporogenous yeasts, grouping them in a family, the Torulaceae. Three genera were recognized: Eutorula, Torula, and Mycotorula. The last is distinguished by rudimentary mycelium, or more precisely by the formation of chains ("sprossverbände") of cylindrical cells. The other two genera present round to elliptical cells. In Eutorula a characteristic development of fat droplets occurs, which is lacking in Torula.

Guilliermond's Classification.—Guilliermond [9] recognized five genera of non-sporogenous yeasts grouped together in a family of

"Non-saccharomycetaceae." Species with lemon-shaped cells are placed in the genus "Pseudosaccharomyces" created by Klöcker to include strains of Hansenia without spores. Two genera are separated on the basis of pellicle formation in liquid media: Mycoderma with thin, dry pellicles, and Medusomyces with thick, gelatinous pellicles. The term Mycoderma has, however, been frequently applied in quite a different sense. In the earlier literature it was used to designate all sorts of organisms forming scums on wine or vinegar, including certain bacteria. Vuillemin applies the term to organisms like *Oidium lactis*, which multiply by the production of oidia or "arthrospores." Because of the different interpretations of the name, its use has become quite ambiguous, and it should be dropped. Following the suggestion of Vuillemin, all pathogenic species are grouped together under the generic name Cryptococcus. This was particularly unfortunate because the character used is one that has not been definitely established for most of the species placed in this genus and is not correlated with any other characters. Moreover, the same criticism of ambiguity will apply; the name was first used to designate certain algae. The remaining yeasts without spores Guilliermond places in the genus Torula.

Classification of Ciferri and Redaelli.—In a monograph on red pigmented yeasts,[4] these authors indicated the lines upon which they erected a new and comprehensive classification of the non-sporogenous yeasts that has just recently been published.[5] Their system is based upon a combination of Will's classification with one proposed by Ota. Their classification includes the yeasts with exogenous spores to be considered later, and also such transitional forms with mycelium as were discussed under the names Oidium and Monilia in the preceding chapter. They have criticised the use of the term Torula on the ground that it has been previously used for a mold in the Fungi Imperfecti, and propose the name Torulopsis for the genus and Torulopsidaceae for the family. But as Harrison [11] has pointed out, the name Torula has been used so uniformly throughout the literature on yeasts, and its other usage is so little known, that on practical grounds it would appear useless to try to replace it.

Omitting the forms with exogenous spores (Nectaromycetaceae) and those with mycelium (Mycotorulaeae), the system of Ciferri and Redaelli is presented below:

Family Torulopsidaceae, Subfamily Torulopsideae

- Cells showing a trace of copulation of germ tubes. *ASPOROMYCES* Chaborski.
- Cells without traces of sexuality.
  - Apiculate cells (lemon-shaped) *KLOECKERIA* Janke.
  - Rounded, oval or elliptical cells, rarely apiculate, and then mixed with the foregoing.
    - Cells normally very small and without a clearly visible double contour. *PITYROSPORUM* Sabouraud.
    - Young cells with one or more fat globules. *EUTORULOPSIS* Ciferri.
    - Young cells without fat globules. *TORULOPSIS* Berlese emend.

The genus Asporomyces contains but a single species which may well be identical with Torulaspora. Kloeckeria is identical with Pseudosaccharomyces in Guilliermond's classification. The name Pityrosporum was applied by Sabouraud to an organism, frequently referred to as the "bottle bacillus," found on the scalp. Its relationships are quite obscure, Ciferri and Redaelli themselves expressing some doubt that it is a yeast. The remaining genera are those of Will, separated on the rather dubious basis of fat globules in the cells.

Harrison's Classification.—Harrison[11] has recently proposed a subdivision of the Torulaceae on almost purely physiological characters which has much to recommend it. He retains the genus Mycotorula of Will for those forms producing rudimentary mycelium. The remaining species are grouped according to pigment production into three genera: Rhodotorula, with red pigment; Chromotorula with pigment other than red; and Torula, with no pigment. Torula and Mycotorula are further divided into groups according to their sugar fermentations as follows:

Group A. No acid or gas in any sugar.
Group B. Slight acid in dextrose, mannose, fructose or galactose.
Group C. Slight acid with or without trace of gas in dextrose, mannose, fructose or galactose and saccharose.
Group D. Marked acidity and gas in dextrose, mannose, fructose, or galactose.
Group E. Marked acidity and gas in dextrose, mannose, fructose, galactose and saccharose.
Group F. Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and raffinose.
Group G. Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and maltose.
Group H. Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and lactose.
Group I. Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose, lactose, and inulin.

A glance at the drawings of three quite distinct Torulae (*d, e, f,* Fig. 85) will indicate the difficulties involved in a morphological classification of this group. *Torula cremoris* is a white species fermenting lactose vigorously and concerned with the gassy fermentation of cream. *Torula glutinis* forms a very characteristic mucoid growth of coral-red color and has practically no fermentative power. *Torula pulcherrima* forms a white growth on solid media with a very striking maroon discoloration of the medium beneath, and produces an alcoholic fermentation of dextrose. And yet under the microscope one finds practically the same range of morphological characters exhibited by all three. This has been the author's experience with Torulae in general. Although certain strains will form small cells, others large ones; some round cells, others elliptical; some with no fat globules, some with a few, others with voluminous vacuoles; yet the variations within a single strain on various media are so great that it would require mathematical analysis of the given characters to clearly differentiate species on such a basis. Therefore the author feels that Harrison's classification is one that is eminently practical.

A large number of specific names have been applied to nonsporogenous yeasts, but because of the difficulties indicated above with regard to classification and bases of identification, these are at the present time almost meaningless. The group is still awaiting its monographist. A beginning has been made by the work of Ciferri and Redaelli and by Harrison, and a line of work for future study has been laid down by Redaelli and Ciferri.[17]

The Torulae are for the most part of no great practical importance. Some strains are a source of trouble in breweries, others are found in dairy products. There are probably several pathogenic species, of which one, *Torula histolytica,* is well defined.

Red Torulae.—These are very common, being encountered frequently as contaminants in cultures in the bacteriological laboratory, and found frequently on a variety of substrates, particularly dairy products. They have been given a variety of specific names, as *glutinis, mucilaginosa, rubra, sanguinea,* etc., which serve to indicate the most striking characters of the group, namely, a sticky or mucoid growth, and a red color. Species have been created on the basis of morphological variations of the cells, i.e., their tendency to form oval or elliptical cells or to produce pseudomycelium; upon differences in consistency of the growth; upon variations in the shade of color produced; and sometimes for no good reason at all. After observing a considerable number of strains of these red yeasts from type culture collections and isolated by myself, the author is still in doubt regarding the unity or plurality of species. Some strains, like the "pink yeast from oysters" isolated by Hunter, have consistently yielded a moist, mucoid growth on solid media, so soft that it runs to the bottom of an agar slant culture. Others have formed cultures which are at first mucoid but gradually become dry and markedly wrinkled on the surface; this change is accompanied by the formation of short strands of pseudomycelium. But one finds so many transitions between the mucoid and wrinkled types of growth that it is impossible to draw lines between the species on this basis. Such morphological differences as occur are within the limits of variability of a single strain. The specific name *glutinis* is apparently the oldest that has been applied to these red yeasts.

Torula pulcherrima is quite different from the common pink to coral-red mucoid yeasts, producing a dark maroon pigment which may color the cells, or more frequently appears only in the substrate. According to Beijerinck,[2] the pigment is secreted as a colorless chromogen which becomes dark red in the presence of iron salts and of air. On agar slant cultures with dextrose one usually obtains a cream-colored growth with a dark maroon zone extending into the agar for some distance below the surface, at times forming a ring in the butt of the tube. But with some brands of dextrose the growth itself becomes darkly colored. This probably varies with the amount of iron occurring as impurities. Very faint traces of iron are sufficient to produce the color. In old cultures the cells develop very large fat globules which distend the cells and change its form from oval to round. Beijerinck states that this yeast may be commonly found on grapes. An apparently identical species has been described by Grosbusch[7] under the name *Torula rubefaciens.*

Yeasts with Exogenous Spores.—The yeasts to be considered under this head are being intensively investigated and their systematic position is still quite uncertain. Both of the two genera produce rudimentary aerial mycelium, and after further study on a variety of substrates it may be found that they are not true yeasts at all, but growth forms of some higher fungi. The two genera are Sporobolomyces and Nectaromyces. The former produces reniform spores on sterigmata which are forcibly discharged at maturity and are therefore considered to be basidiospores. The latter forms globular bodies on short stalks formed in whorls as in Verticillium; these are looked upon as conidia.

Sporobolomyces.—A member of this genus was described and clearly illustrated by Fischer and Brebeck, but the peculiar characters of the group were first recognized by Kluyver and van Niel.[13] If one inoculates a Petri plate culture with this organism in a characteristic pattern and incubates the plate in an inverted position, there is formed a faint duplicate of the colony pattern upon the lid of the dish (Fig. 87). These "mirror colonies" are formed by a deposition of the spores which are discharged from the surface of the colony.

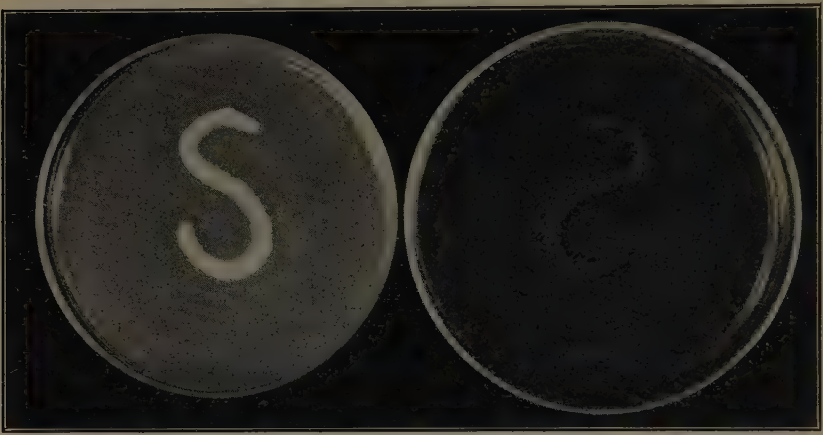


FIG. 87.—*Sporobolomyces salmonicolor.* Petri plate culture showing development of "mirror colony" on the lid.

Microscopic examination of a young colony shows only the presence of ordinary oval, budding, yeast cells. But later, as spores develop (which is manifested by the occurrence of a powdery coat on the surface of the colony), many cells will be found with fine pointed projections exactly like the sterigmata which are found on the basidia of a mushroom. On these there appear typical kidney-shaped cells which reproduce very closely the form of the basidiospores of some mushrooms (*s*, Fig. 88). The discharge of these spores has been observed by Kluyver and van Niel and by

Buller,[3] who report a mechanism which exactly parallels that in the Basidiomycetes. The discharge of the spore is accompanied by the development of a drop of water at the point of attachment of the spore with its sterigma. When this drop has reached a certain size, the spore is suddenly and forcibly propelled into the air. Buller * has observed the successive production and discharge of as many as three spores from one sterigma, and assumes that four may be formed. Occasionally more than one sterigma may form from a single cell.

Guilliermond [10] has studied the cytology of this yeast, and finds but a single nucleus in a cell, with no evidences of conjugation. On this basis he concludes that the spores are not to be considered as basidiospores, but rather as conidia, a conclusion accepted by Ciferri and Redaelli.[5] But, to be consistent, he would then also have to conclude that the endogenous spores formed by other yeasts in which no trace of conjugation occurs cannot be accepted as ascospores, although Guilliermond himself has very carefully traced the loss of sexuality and development of parthenogenesis, in the spore-forming yeasts. The form, mode of production, and discharge of the spores are so constant in the Basidiomycetes and so definitely absent in other groups of fungi that it may safely be accepted as a criterion of the class, and we are therefore justified in accepting Sporobolomyces as a yeast derived from the Basidiomycetes, perhaps so degraded in its evolution as to have completely lost all traces of sex.

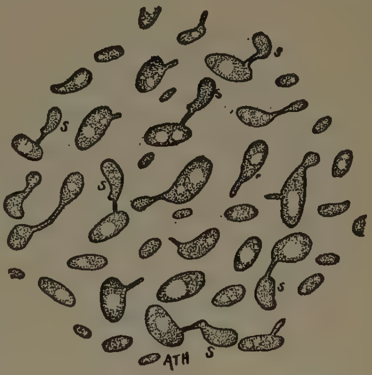


FIG. 88.—*Sporobolomyces salmonicolor*. Basidiospores attached to sterigmata are marked *s*.

Kluyver and van Niel described three species, *S. salmonicolor*, *S. roseus*, and *S. tenuis*. All three produce pigment of various shades of rose. Buller has observed further species, and finds such yeasts frequent upon straw.

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* Personal communication.

Nectaromyces.—These yeasts have been recently studied by Gruess [8] and by Nadson and Krassilnikov.[15] Gruess gave them the generic name Anthomyces. But, according to Ciferri and Redaelli,[5] this name was previously applied to a fungus belonging to the rusts, and therefore they accept the name Nectaromyces given later by Schoellhorn. But one species is known, *Nectaromyces reukaufii.*

These yeasts are found in the nectar of flowers, in which they appear characteristically in groups of four (occasionally more) long, slender cells. These are arranged somewhat like an airplane with its outspread wings. The investigation of Gruess indicates that these "airplane forms" are produced by a growth in media of low nutrient value. In ordinary sugar media as used for yeasts, only large oval budding forms develop. But if the concentration of nutrients is greatly reduced, the form characteristic of the nectar growth also appears in artificial cultures. Nadson and Krassilnikov suggest that the airplane form is an adaptation which has resulted from natural selection, enabling the yeast to adhere easily to the hairs on insects and so be transported to new flowers.

The latter authors observed sectorial mutations to occur in giant colonies, in some of which short filaments of true mycelium occurred which developed globular conidia on slender lateral branches appearing much as in the mold Verticillium. They also made cytological studies and found but a single nucleus in all of the various growth forms.

Identification of Yeasts.—In the present state of our knowledge, it is frequently very difficult, sometimes impossible to identify unknown yeasts. It is relatively easy to place a yeast in its genus, especially if spores are formed. But so many species have been described, often inadequately, that it is often impossible to decide whether one has to deal with a form that has been previously recorded or a new one. Certain species, of course, have such striking characters that they may be identified at once, such as *Willia anomala*; others, notably members of the genera *Zygosaccharomyces*, *Saccharomyces*, and *Torula*, present so many intergrading types that one is quite at a loss in identifying unknown strains.

Strains of yeasts known to have formed spores may frequently fail to produce them on subsequent trials, no matter what method is used to stimulate their production. Such experiences make one hesitate to refer an unknown yeast definitely to the non-sporogenous family.

Many yeasts are rather variable in some of their characters, and there have been reported several apparently distinct mutations. These appear especially in giant colonies, either as variant sectors or as secondary colonies; when such areas are subcultured, new races are obtained which breed true.

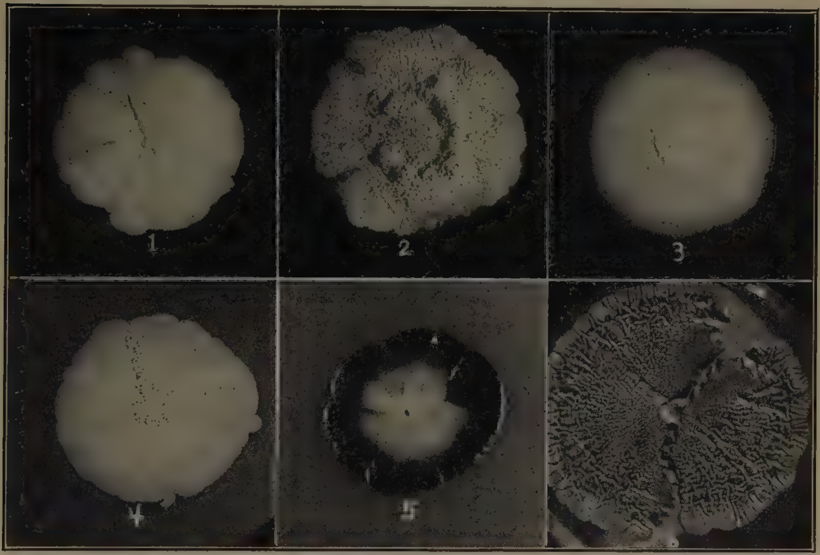

FIG. 89.—Giant colonies of some yeasts. 1. *Saccharomyces* species from soil. 2. *Pichia membranafaciens.* 3. *Willia anomala.* 4. *Torula cremoris.* 5. *Torula pulcherrima.* 6. *Torula glutinis.*

The use of giant colonies, introduced by Lindner, as a means of differentiating species has proved disappointing because of the marked variability occasioned by even slight changes in the composition of the medium. These colonies present surface patterns which are of the same general character as those designated "smooth" and "rough" in bacterial colonies. But as Lindner himself showed, the texture of the colony may be markedly varied by changing the consistency of the gelatine used. The author has found that the appearance of the colony may be quite different if a highly purified dextrose is substituted for a commercial crude dextrose. Until a perfectly standardized medium can be developed, it would seem that the giant colonies can be looked upon only as a general guide, not as a means of specific differentiation.

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CHAPTER IX

BIOLOGICAL ACTIVITIES OF THE YEASTS

The yeasts are of importance principally, of course, because of the alcoholic fermentation which they produce. We are well acquainted with a few species, generally designated as cultivated yeasts, which have been used since time immemorial for the making of beers and wines, and in baking. A number of other species which have been used in various kinds of alcoholic drinks have also been described. But aside from these there exists a rather large number of species of yeasts which do not form alcohol, or at least not in sufficient amounts to be of commercial value. A number of these have been described, mainly contaminants in alcoholic fermentation. A few species are pathogenic to man, and the pathogenic properties of a number of others have been experimentally investigated.

It was Pasteur who first proved what had been previously suspected, that yeasts are the cause of the alcoholic fermentation of sugary solutions. It was he, also, who showed that failures in brewing were due to contamination with other organisms. These undesirable fermentations Pasteur believed were due entirely to bacteria; to prevent them he introduced the process of heating the beer-wort to a temperature that would kill or inhibit bacteria, but low enough so that it would not injure the flavor or foaming properties. This process, later applied to milk, has come to be known as pasteurization. But the undesirable fermentations in beer are more frequently due to wild yeasts than to bacteria, and these may be carried over in the "starter" and give rise to endless trouble. This was the discovery of a Dane, Emil Christian Hansen, and the early development of our knowledge of the nature of yeasts and their relationships, as well as the introduction of scientific methods, and use of pure cultures, etc., in the fermentation industries are largely due to him and his pupils at the Carlsberg laboratory in Copenhagen.

After Pasteur had demonstrated that fermentation was caused by yeasts, it was for some time believed that fermentation was a strictly vital process, i.e., it could be brought about only by the living protoplasm, because no fermentation was brought about by the fluid in which the yeasts were growing, but from which they had been filtered out. The classical experiment of Buchner demonstrated that this is not the case, but that fermentation is due to an enzyme normally retained within the cell (endoenzyme) which can be expressed in the cell sap by hydraulic pressure. This experiment made it possible to study fermentation by itself, i.e., aside from the other chemical processes of the cell, and at least partially to purify the enzyme and determine something of its chemical nature. Of the large amount of investigation of the chemistry of alcoholic fermentation which has appeared, the work of Harden and Young stands out.

Chemistry of Alcoholic Fermentation.*—The production of alcohol from sugar, known since earliest antiquity, has long been of interest to chemists. It was one of the first reactions demonstrated to be due to an enzyme formed by living cells, and has been more intensively investigated than any of the other enzyme reactions.

Earlier Chemical Theories.—As chemical science emerged from the mists of alchemy, definite ideas about the nature of alcoholic fermentation began to be formed. From 1660 to 1790 chemists who studied this problem had concerned themselves chiefly with the study of the gases that were liberated in the process of fermentation, but it was not until the time of Lavoisier (1789) that any quantitative studies were made of the products of fermentation. At this time Lavoisier showed by quantitative analysis that all the carbon, hydrogen and oxygen of the fermented sugar could be accounted for in the products, alcohol and CO_2. This made it appear to the chemists of those days that the phenomenon of fermentation belonged to the realm of pure chemistry, since it obeyed the quantitative laws of other known chemical reactions. From 1790 until 1885 chemists generally maintained that the reactions involved in fermentation were purely chemical and not biological in nature. During this period there were a few men who from rather careful study arrived at the conclusion that the process was biological in nature and that yeasts were actually living cells. The most prominent of these were Cagniard-Latour, Theodore Schwann and Kützing. The conclusions drawn by these three men, however, were bitterly opposed by the prominent chemists of the times, and their views had little effect on the teachings of the period. Prominent among the supporters of the chemical theory of fermentation were Gay-Lussac and Thenard in the earlier part of the period and Berzelius and Liebig in the latter part of the period. The most ardent supporter of the chemical theories was Liebig. His theories were accepted by everyone as the correct explanation of the phenomena of fermentation. Liebig assumed that ferments were not living cells but merely organic catalysts that were in a state of extreme disquietude. This state of disquietude was transferred to the sugar molecules, which were then changed into forms more stable, namely into alcohol and carbon dioxide.

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* This section has been prepared in collaboration with Dr. H. O. Halvorson, University of Minnesota.

The Liebig-Pasteur Controversy.—The classical experiments of Pasteur which finally proved that yeasts are the cause of alcoholic fermentation began in 1857. After showing that the lactic acid fermentation was due to living microorganisms (bacteria), he applied the same experiments to alcoholic fermentation. Liebig's theory of the origin of yeasts by the action of the oxygen of the air on the nitrogenous matter of the fermentable liquid was conclusively disproved by the simple device of producing a crop of yeast in a liquid medium containing only inorganic materials such as sugar, ammonium tartrate and mineral phosphates. Here there was obviously present in the original medium, no matter which could be put into a state of putrefaction by contact with oxygen and extend its instability to the sugar. Any such material must first be formed by the vital processes of the yeast. Further, Pasteur showed that, wherever fermentation occurred, growth and multiplication of the yeasts accompanied the phenomenon. The sugar, he proved by careful analysis, was not completely decomposed into alcohol and carbon dioxide, as was claimed by Lavoisier. A small amount of the sugar was always unaccounted for. This Pasteur showed must appear as protoplasm of the yeast. When Pasteur finally offered to grow yeasts on chemicals furnished him by Liebig, the latter had to give up his chemical theory of fermentation in favor of the biological theory, and thereby the voice of the last adherent to the chemical theory was stilled.

Fermentation a Vital Phenomenon.—Traube, in 1858, first suggested that all fermentations are due to substances secreted by living cells. Pasteur attempted to prove this hypothesis with yeasts. He therefore tried by all conceivable means to find the ferment produced by the yeast cells. His attempts as well as the attempts of numerous other investigators from 1860 until 1897 were failures. These results finally led Pasteur to the view that yeasts do not form ferments and that fermentation in this case represents life without oxygen. He came to look upon fermentation as a method by which yeasts obtained energy for growth and that the fermentation was a part of the vital phenomenon that could not be separated from the life of the yeast. He called it life by means of intramolecular respiration. By 1897 this explanation was quite generally accepted.

Discovery of Zymase.—It was quite by accident that the fallacy of this view was discovered. As early as 1893 Hans and Edward Buchner found that the cells of even the smallest microorganisms could be broken by being ground with sand, and in 1896 the same process was applied by these investigators to yeasts, with the object of obtaining a preparation for therapeutic purposes. To obtain the intra-cellular fluid they subjected the ground cells to pressure in a hydraulic press. The yeast juice thus obtained underwent changes very rapidly. The ordinary antiseptics were found to be unsatisfactory in preserving the juice, hence sugar was finally added as a preservative. The marked action of the juice upon the sugar drew Edward's attention to the fact that fermentation was proceeding in the absence of yeast cells.

In his first papers Buchner established the following facts: (1) Yeast juice free from cells is capable of producing the alcoholic fermentation of glucose, fructose, cane sugar and maltose. (2) The fermenting power of the juice is not destroyed by the addition of chloroform, benzene, or sodium arsenate, by filtration through a Berkefeld filter, by evaporation to dryness at 30° to 35° C., nor by precipitation with alcohol. (3) The fermenting power is completely destroyed when the liquid is heated to 50° C. From these facts he drew the conclusion that the production of alcoholic fermentation does not require so complicated an apparatus as the living yeast cell, and that the fermenting power of yeast juice is due to a dissolved substance. To this active substance he gave the name *zymase.*

Further studies have proceeded along two distinct lines. On one hand the physiology of yeasts has been investigated with a view to confirming or disproving Pasteur's theory of intramolecular respiration. Other studies have been concerned with the nature of the zymase itself and the chemical reactions which it causes.

Intramolecular Respiration.—It is obvious, if Pasteur's theory were correct, that yeast cells should be able to grow as well in the absence of atmospheric oxygen as in its presence. If yeasts obtain energy for growth by changing sugar into alcohol and carbon dioxide, they do not need free oxygen. It was soon learned that yeast cells do not grow as well under anaerobic conditions as under aerobic conditions. This would appear to indicate that Pasteur's theories are incorrect, but the argument still remains that, when yeast cells are forced to grow in anaerobic or semi-anaerobic conditions, they get at least a portion of their energy by the process of intramolecular respiration. This point remained controversial until 1924. Up to this time all researches along this line were attempts to show that yeast cells would or would not grow in the absence of oxygen. Due to the fact that some investigators reported good growth of yeast under such conditions, while others reported no growth, the question remained in dispute. The growth obtained by some men could always be explained on the basis of the incomplete removal of oxygen.

The researches of Brown and Balls [2] in 1924 appear to disprove Pasteur's theory. By very careful analysis they have correlated the disappearance of sugar, the production of alcohol and carbon dioxide with the rate of growth of yeast cells. These quantitative studies of yeasts in the presence of oxygen, together with those of Slator on the rate of fermentation by yeast cells in the absence of oxygen, seem to justify the following conclusions:

1. The rate of disappearance of sugar and of appearance of carbon dioxide is definitely related to the growth of the yeast cells when oxygen is supplied so that it is not the limiting factor.
2. The rate of formation of alcohol is in no way related to the growth of the yeast cells when oxygen is not supplied, but the rate of formation of alcohol is directly proportional to the number of yeast cells present, even though the growth rate is zero. The rate of fermentation per cell is no greater if the rate of growth attains some positive value.

3. The amount of alcohol that can be obtained from a given amount of sugar decreases as the rate of yeast growth increases.

The above conclusions are the basis for the following views:

1. Yeast cells do not obtain energy for growth by the reactions which change sugar into alcohol and carbon dioxide.
2. The phenomenon of fermentation is a side reaction that occurs in the chain of reactions which are a part of the metabolism of sugar by yeast cells, and this reaction is of no benefit to the yeasts.
3. Yeast cells obtain energy for their growth only by the oxidation of sugar or other compounds completely to carbon dioxide with the aid of atmospheric oxygen.

The Nature of Zymase.—A voluminous literature has appeared dealing with the chemistry of zymase and the reactions which it initiates. Prominent workers in this field are Buchner, Euler, Fernbach, Harden and Young, Lebedev, Neuberg, and Slator. The paragraphs below present a general summary of their investigations, in which no attempt is made to present the work of each author separately.

Preparation of Zymase.—There are several methods now available whereby zymase may be prepared free from living yeast cells. It can be prepared as Buchner did, by grinding the living yeast cells with sand until the mass becomes semi-liquid in consistency and then separating the liquid from the sand and cells by a hydraulic press. The zymase will be in the extract so obtained.

An active preparation may also be obtained by dehydrating some yeast cells and then pulverizing the dried cells. When this is again macerated in water, the enzyme goes into the solution. A similar preparation may be obtained by dehydrating the cells with acetone and then pulverizing them. A preparation made in this way is called "zymin." Attempts to purify the enzyme have not been successful. None of the enzyme preparations have as strong a fermenting power as the living yeast cells. Of the various preparations, zymin is the strongest, Buchner's the poorest.

Enzyme and Coenzyme.—In the earliest work on zymase it was discovered that the enzyme could be separated into two parts. This separation can be effected by means of a semi-permeable membrane. One part passes through the membrane, the other does not. That part which filters through the membrane is called the coenzyme. It has been found to be thermostable and seems to be inorganic in nature. The nature of the compound is not known but it apparently contains some phosphorus. That part which does not diffuse through the membrane is called the enzyme proper. It is thermolabile and appears to be a protein in nature. Both of these substances are essential for fermentation to take place. During fermentation both of them disappear slowly. If the coenzyme is present in the smallest amount, fermentation can again be initiated by adding some more coenzyme. If the enzyme proper is present in the smallest amount, the same result is obtained by adding more of the enzyme proper. Both of them break up most rapidly in the absence of sugar, but both are stable in a dry state. The enzyme proper can be obtained only by separating it from the coenzyme by means of a semi-permeable membrane. The coenzyme can be prepared by heating a solution containing both, so as to destroy the enzyme proper.

Phosphates Necessary for Fermentation.—It was believed that the enzyme disintegrated because of the presence of proteolytic enzymes that decomposed the zymase. Attempts were made to prepare an anti-enzyme that would prevent this action by injecting zymase into animals and then using the serum of these animals as the antiferment. These experiments led to the discovery that, when any serum is added to a fermenting mixture, the rate of fermentation is increased for a time. After trying many chemical compounds to see what ingredient of the blood possessed this property, it was found that the same effect could be obtained by adding any soluble phosphate to the fermenting solution. These experiments resulted in the discovery that not only would phosphate increase the amount of fermentation, but unless phosphates are present no fermentation can take place even though both coenzyme and the enzyme proper are present. It was further found that, when a certain amount of soluble phosphate is added to the fermenting solution, the rate of fermentation is speeded up until an extra molecule of alcohol is formed for every molecule of phosphate added; after that the fermentation proceeds at the normal rate.

Reactions of the Phosphates.—Harden and Young have investigated this quantitative relationship and have been able to show rather definitely what function the phosphates play. They found that, when any soluble phosphate is added, it is at once changed into some organic phosphate having the formula $H \cdot PO_4 \cdot R_2$. This compound in the presence of zymase reacts with one molecule of sugar to form hexose monophosphate.

$$(1) \quad C_6H_{12}O_6 + HPO_4R_2 \text{——} C_6H_{11}O_5PO_4R_2 + H_2O.$$

In the presence of zymase, two of these compounds react with each other to form a hexose diphosphate and methylglyoxal.

$$(2) \quad 2C_6H_{11}O_5PO_4R_2 \text{——} C_6H_{10}O_4(PO_4R_2)_2 + 2CH_3 \cdot CO \cdot CHO + 2H_2O.$$

In the presence of zymase, the hexose diphosphate breaks down to form sugar and the organic phosphate.

$$(3) \quad C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O \text{——} C_6H_{12}O_6 + 2PO_4HR_2.$$

The phosphate formed can now enter reaction (1) again. When a soluble phosphate is added to a fermenting solution, reactions (1) and (2) take place rapidly, which accounts for the increased amount of fermentation since methylglyoxal is changed to alcohol and carbon dioxide rapidly. Reaction (3) is slower, however; therefore when all the phosphate has been used up the only phosphate available for reaction (1) and for the production of alcohol is that liberated by equation (3). If reaction (3) could be speeded up, it would accelerate the rate of fermentation. It has been found that arsenates and arsenites will do this. If small amounts of these compounds are added to the fermenting solution, the rate of fermentation is increased considerably. In this case, since phosphate is being formed more rapidly, addition of phosphates should have little effect upon the rate of fermentation. This has been proven to be the case. It has also been found that additions of phosphate do not accelerate the rate of fermentation brought about by living yeast cells. It is believed, therefore, that in living yeast cells the hexose diphosphate decomposes faster than in zymase preparations. This fact has been used to explain why zymase always has a lower fermenting power than living yeast cells.

Fermentable Carbohydrates.—Zymase is able to ferment glucose, fructose, mannose and galactose. Of these four sugars fructose is fermented most rapidly. It is believed that fructose is the only sugar that is fermented directly, and that the other three sugars are changed to fructose by an enzyme before they can be fermented. The other sugars are fermented more slowly because they are converted to fructose slowly. If any substance could be added which would cause them to be changed to fructose rapidly, glucose, mannose and galactose would be fermented just as rapidly as fructose. It has been found that small amounts of toluene will do this. Small amounts of toluene added to a fermenting solution containing glucose or galactose will speed up the rate of fermentation so that it approaches the rate at which fructose is fermented. Toluene has no effect upon the rate of fermentation of fructose.

Yeasts cannot ferment the polysaccharides. To produce alcohol from starch, for instance, one must first provide a diastase from some other source than the yeast, to convert the starch to maltose.

On the other hand, most yeasts can ferment the disaccharides sucrose and maltose, and a few also lactose, as was indicated in the preceding chapter. But these disaccharides must be first broken up into their component monosaccharides by appropriate enzymes, which are independent of zymase. Thus invertase is formed by most yeasts to convert sucrose to glucose and fructose; maltase changes maltose to glucose; and lactase splits lactose into glucose and galactose.

Next, as was pointed out above, probably both glucose and galactose are converted to fructose before they are fermented, and for this two further enzymes are required. The nature of the chemical reaction involved is not known. Most yeasts ferment galactose very slowly or not at all.

Zymase a Battery of Enzymes.—Thus it will be seen that a whole series of enzyme reactions are necessary before the alcoholic fermentation proper can be started. But there is considerable evidence to indicate that the zymase itself consists of several different organic catalysts. There is much to indicate that it contains a carboxylase, which splits off a —CO_2 group from certain organic acids formed in the chain of reactions.

There must also be in zymase an enzyme which can bring about oxidation and reduction reactions. An organic sulphur compound called glutathione, which possesses this ability, has been isolated from yeast cells and it is believed that this compound is responsible for the oxidations and reductions that take place in the chain of reactions that are part of the fermentation. This compound will react with a molecule of water and add the H— of the water to one compound, thereby reducing it, and add the —OH group to another compound, thereby oxidizing it.

The Alcoholic Fermentation Proper.—The following statements are intended to give a comprehensive picture of the chemistry of alcoholic fermentation, combining the observations of Harden and Young on the relation of phosphates to the process, with Neuberg's pyruvic acid theory.

The first step in the alcoholic fermentation proper, which takes place through the influence of zymase, is the formation of a hexose monophosphate (equation (1), above) by combining the fructose with an organic phosphate.

The zymase acts still further on this hexose monophosphate to produce hexose diphosphate and methylglyoxal (equation (2), above).

The hexose diphosphate may be then broken down to form fructose and phosphate (equation (3), above) which are again available to start the reaction over again. This reaction is also due to zymase.

Under the influence of the oxidizing and reducing activities of glutathione, the methylglyoxal goes through a series of reactions which finally yield alcohol and carbon dioxide, together with some glycerin, as follows:

$$(4)\quad \underset{\text{Methylgloxal}}{2CH_3\cdot CO\cdot COH} + 2H_2O \text{——} \underset{\text{Glycerin}}{CH_2OH\cdot CHOH\cdot CH_2OH} + \underset{\text{Pyruvic acid}}{CH_2:COH\cdot COOH}$$

The glycerin resulting from this reaction remains as one of the end-products of the fermentation.

The pyruvic acid, through the action of carboxylase from the yeast, forms ethylaldehyde and carbon dioxide.

$$(5)\quad \underset{\text{Pyruvic acid}}{CH_2:COH\cdot COOH} \text{——} \underset{\text{Ethylaldehyde}}{CH_3\cdot CHO} + CO_2$$

The carbon dioxide resulting from this reaction remains as one of the end-products of fermentation.

The ethylaldehyde formed enters into a reaction with methylglyoxal, again under the combined oxidizing and reducing action of glutathione, yielding more pyruvic acid and alcohol, thus:

$$(6)\qquad \begin{aligned} &\underset{\text{Methylglyoxal}}{CH_3\cdot CO\cdot COH} + \underset{\text{Ethylaldehyde}}{CH_3\cdot CHO} \\ &\qquad + 2H_2O \text{———} \underset{\text{Pyruvic acid}}{CH_2 : COH\cdot COOH} \\ &\qquad + \underset{\text{Alcohol}}{CH_3\cdot CH_2\cdot OH} + H_2O \end{aligned}$$

The alcohol, of course, remains as an end-product of the fermentation, whereas the pyruvic acid is free to enter reaction (5) again. Once this reaction has taken place, therefore, reaction (4) is no longer necessary for a continuation of the fermentation.

It will thus be seen that a whole series of catalysts is required for the production of alcohol. If, for instance, alcohol is formed from maltose, there is required *maltase* to form glucose, an unknown enzyme to change this to fructose, *zymase* to carry on reactions (1), (2), and (3), *glutathione* for reactions (4) and (6), and *carboxylase* for reaction (5). It is not known what part the enzyme proper and the coenzyme of zymase play respectively in reactions (1), (2) and (3). It is probable that here too we are dealing with a mixture of catalysts.

Production of Glycerol.—It will be noted that, if the pyruvic acid could be removed from the spheres of the reactions as fast as it is formed, no ethylaldehyde would be formed; therefore reaction (5) would be cut out and no alcohol would result. Instead, reaction (4) would continue, giving rise to glycerin. This can actually be accomplished by adding sodium sulphite to the fermenting solution. The sodium sulphite either removes the pyruvic acid as fast as it is formed, or it interferes with the action of the carboxylase and, as a result of this, glycerin is formed at the expense of the alcohol. This fact has been made use of industrially in the use of yeasts for the production of glycerin, particularly in Germany during the war when the normal supply of glycerin from fats was cut off. It is said that as much as 27 per cent of the sugar may be thus converted to glycerin.

The Function of Alcoholic Fermentation.—Although some of the yeasts can apparently utilize alcohol to a small extent as a source of food, this is not the case with the majority of species. The production of alcohol, as was pointed out above, is not a part of the normal nutrition of the organism. There has, therefore, been some speculation as to what purpose it serves in the economy of the yeast. Wortmann and Delbrück point out that yeasts can tolerate more alcohol than most other organisms, and that by producing alcohol in sugar solutions they are able to get rid of competing organisms, molds and bacteria.

Symbiotic Fermentations.—Although it is true that yeasts must compete with bacteria (which generally grow more rapidly) in most environments, they are found growing together in a symbiotic relationship in certain situations. Thus the ferment of kefir is composed of a yeast and a bacterium (*Streptococcus kefir*). Freudenreich, who studied this ferment, claims that the yeast is unable to ferment lactose, but that the milk sugar is first inverted by the streptococcus, the resulting hexoses being changed to alcohol and carbon dioxide by the yeast. A similar relationship is present in the ginger beer "plant" and in a curious ferment which has been used in rural America for the manufacture of vinegar directly from molasses. In the latter ferment the bacterial symbiont is apparently an acetic bacillus. The yeast first produces alcohol from the sugar; then the bacillus oxidizes the alcohol to acetic acid. In all three of these ferments the organisms grow together as curious irregular lumps of cartilaginous consistency, part of the sugar being converted into a gum which holds them together. These masses or grains are strained out after the fermentation is complete, and will remain viable after drying, for considerable periods of time.

Production of Fat by Yeasts.—In his studies of *Torula pulcherrima* Beijerinck suggested that the abundant development of fat globules might have commercial possibilities, but abandoned the idea because the fat was not produced abundantly save on solid media. Nadson and Konokotine[21] have also investigated the possibility of producing fats from yeasts, and more particularly from a species of Endomyces. The chemistry of fat production by yeasts has been extensively studied by MacLean and Hoffert.[17, 18, 19] Nothing practical has, however, developed in this field.

Occurrence of Yeasts in Nature.—We find yeasts free in nature wherever there is sugar present, in the various foodstuffs of man, in the nectar of flowers, the exuded sap of trees, and above all on the surfaces of fruits. They are also found in soil, on the leaves of plants, and as symbionts or parasites in various animals, especially insects.

The constant occurrence of yeasts on fruits, especially grapes, naturally led to inquiries as to their origin. Pasteur showed that the immature fruits are free from yeasts, and that, if they are covered with cotton while ripening, no yeasts will develop. Hansen believed, as a result of investigations with *Hansenia apiculata*, that the yeasts remain dormant in the soil during the winter and spring, probably in the form of ascospores, and are deposited on the fruit by the wind, the spores there germinating and budding, to form new spores on the overripe fruit at the approach of winter. He found yeasts much more numerous in the soil of vineyards than elsewhere. But Starkey and Henrici [29] have found yeasts to be relatively infrequent in soil, being present in very small numbers in only 38 of 87 soil samples of all kinds, including a number from orchards; and the varieties found were for the most part not those kinds found to be active in the spontaneous fermentation of fruit juices, only four cultures of Saccharomyces having been obtained. Yeasts have been found very frequently on insects and in their digestive tracts, and it is probable that insects are more important in distributing yeasts on fruits than is the chance distribution of infrequent yeasts from soil by the wind.

Yeasts in Nectar and Honey.—Aside from the peculiar yeast, *Nectaromyces reukaufii*, referred to in the preceding chapter, numerous other yeasts have been found in the nectar of flowers. These have recently been studied by Zinkernagel,[38] who presents a summary of previous literature. Zinkernagel described fifteen species obtained from various flowers, none of which formed ascospores. Lochhead and Heron,[15] studying the fermentations of stored honey, found these to be due in every case to yeasts of the genus Zygosaccharomyces, which could not only grow in high concentrations of honey but failed to develop in media of low osmotic pressure. Thus in tubes containing 10, 25, 50, 65 and 75 per cent of honey, fermentation occurred in only the first three with *Saccharomyces ellipsoideus*, while it occurred only in the last three with a yeast from fermented honey. An investigation of the nectar of flowers showed the occurrence of similar yeasts of high sugar tolerance belonging to the Zygosaccharomyces group.

Yeasts and Insects.—Since yeasts are apparently distributed in nature largely through the agency of insects, it is not surprising to find yeasts frequently present in the alimentary tracts of these animals. Thus Berlese believed that the intestinal tract of

Diptera was the normal habitat of many yeasts, including *Saccharomyces ellipsoideus.* It has more recently been shown that the yeasts form an important part of the diet of certain insects, such forms as the fruit flies (Drosophila) for instance, depending for their nutrition not so much upon the fruit as upon the yeasts growing there. In some insects, however, the yeasts penetrate the body cavity and live there, probably as symbionts, since they apparently do no harm to the insect, and are invariably present, being transmitted in the ova. These symbiotic yeasts are found particularly in the Homoptera, especially the cicadas, and are contained in a special mass of tissue, the pseudovitellus, so that microscopically they appear at first glance as some sort of a gland. The species found are for the most part members of the genus Schizosaccharomyces. They have been especially studied by Sulc. In another Homopterous insect, the locust, there occurs a pathogenic yeast which invades the blood and multiplies sufficiently to make the blood a milky white color instead of its normal clear straw color. It kills the insect, and can be transmitted by inoculation. We have already noted the occurrence of a peculiar parasitic yeast, Coccidiascus, in the intestines of the fruit fly, *Drosophila funebris.*

Yeasts in Food Products.—In foods preserved from bacterial decomposition by high acidity or high osmotic pressures, yeasts as well as molds may grow. But unlike the molds, many yeasts can grow under semi-anaerobic conditions. For the most part, yeasts are not at all active in the decomposition of proteins. They become undesirable because of their active fermentation of carbohydrates with resulting gas production. In some cases they probably act as does *Oidum lactis*, upon organic acids, reducing the acidity of the medium to a point where putrefactive bacteria may grow.

Yeasts become a serious problem in the preservation of fruit juices, whose flavor is impaired by heating or by adding preservatives. Vandecaveye[36] has described a unique method for preventing yeast growth in cider. The cider is inoculated with yeast and incubated until the first signs of fermentation appear; it is then heated sufficiently (45° for 30 to 45 min.), to inhibit the fermentation; reinoculated, incubated, and heated, until three treatments have been given. According to Vandecaveye this procedure allows the yeasts to use up most of the available nitrogen and phosphorus in the apple juice, so that no further yeast development occurs. Treatment with an adsorbent (agar) to aid in clearing, and the addition of a minute trace (0.04 per cent) of sodium benzoate are desirable additional procedures.

Dairy Yeasts.—Yeasts are extraordinarily abundant in various dairy products, notably cream, butter and cheeses. Yeasts occur quite regularly in cream, and the numbers rise considerably when the cream sours. They are naturally then abundant in butter. In some cheeses, as Camembert, yeasts make up a large proportion of the microbic flora.

The yeasts found in dairy products are for the most part Torulae, though an occasional sporulating yeast has been isolated. A tentative classification of these dairy Torulae has been proposed by Cordes and Hammer,[6] who make a primary subdivision into those with colored (mostly red) colonies, and those with white colonies. The latter are subdivided into species with dull, irregular colonies and those with smooth, regular colonies, and the last group is separated into those producing gas from milk (*Torula cremoris*, *Torula sphaerica*) and those not fermenting milk. This final group contains a large number of forms grouped together under the name of "common white yeasts." Nelson [22] has suggested a further subdivision of these on the basis of temperature relations and action on milk, into four types.

Lactose fermenting Torulae occasionally give considerable trouble in cream, producing a marked gaseous fermentation known as "foaminess." Foamy cream is due, according to Hammer and Cordes,[12] to two species of yeasts, *Torula cremoris* with large oval cells growing best at 37° C., and *Torula sphaerica* with small round cells growing very poorly at 37° C. These are constantly present in cream, and the fermentation can be prevented only by proper cooling.

The occurrence of yeasts, together with molds, in butter has been rather extensively investigated recently. Macy and Ritchie[16] found yeasts to occur in numbers as high as 90,000 per cubic centimeter. The yeast count in general correlated with the mold count, but neither could be definitely correlated with the keeping quality of the butter.

Harrison [14] has studied the occurrence of Torulae in cheeses (presumably Cheddar) and has described a number of species. Although yeasts were found in all cheeses examined, the presence of large numbers was associated with off flavors causing the cheese to be graded down. These off flavors are attributed to the production of esters by the yeasts.

Normal Occurrence of Yeasts in Man and Animals.—Higher animals consume yeasts with their food, and these organisms have frequently been demonstrated in the alimentary tracts of animals and man. *Saccharomycopsis guttulatus* is apparently a constant normal parasite of the intestines of rabbits. Most of the species found in higher animals are, however, undoubtedly transitory organisms, not multiplying in the body. Anderson[1] has demonstrated that most species of yeasts are not injured by the digestive juices, but pass through the alimentary tract, and has described a number of species from human feces. Rettger, Reddish and MacAlpine,[28] however, found that baker's yeast was rapidly destroyed in the alimentary tract. The occurrence of yeasts in the normal throat has been studied by Tanner, Lampert and Lampert.[34] They did not, however, clearly distinguish the strains they isolated from Moniliae. Yeasts were found in 10 per cent of the throats examined. Eighteen of forty-seven strains were pathogenic to mice. The frequent occurrence of yeasts in or on the human body has not been sufficiently considered by the various authors who have described pathogenic yeasts.

Pathogenic Yeasts.—It is very difficult to analyze the extensive literature on yeast infections in man. Many of the organisms reported have clearly not been yeasts, but parasites of the type of *Oidum dermatitidis* or *Monilia albicans,* forming mycelium either in the lesions or in cultures. In many other cases the organisms have been so incompletely studied or described that it is impossible to determine whether the author was dealing with a true yeast or with a yeast-like form of a mycelial fungus. A large number of cases have been reported in which the pathogenicity of the organism has been very imperfectly established, the mere presence of the yeast being considered sufficient evidence of its etiologic relationship to the disease. Where these organisms have occurred alone in deep-seated lesions, such evidence may be given weight. But in the numerous cases where yeasts have been found in superficial skin lesions, in the mouth and throat, sputum, or feces, associated with numerous other organisms, their presence can hardly be considered as evidence that they are the cause of the disease process under consideration. Even pathogenicity for laboratory animals, unless pronounced, should be considered guardedly, since various wild yeasts have been found to produce abscesses in lower animals when inoculated subcutaneously.

Yeasts in Tumors.—Considerable interest in the pathogenic properties of yeasts was manifested some years ago when it was claimed by several authors, particularly Sanfelice, that cancer was caused by these organisms. This theory developed from the observation of round hyaline masses bearing some resemblance to yeast cells in the cells of the tumors. These we now know are not intracellular parasites of any kind, but are the result of degenerative changes in the cells. But strength was given to this hypothesis by the repeated cultivation of yeasts from tumor tissues and by the production of tumor-like inflammatory masses when these were inoculated into animals. The yeasts obtained from cancers, however, are not pathogenic to man and are only occasionally present on the ulcerating surface of the tumor as accidental contaminants, whereas the tissue reactions in the inoculated animals bear only a superficial resemblance to a true tumor.

Classification of Yeast Infections.—In clinical literature yeast infections are almost universally referred to as " blastomycoses," which is doubly misleading, because yeasts are not "Blastomycetes" and because the name is so generally applied to infections by *Oidium dermatitidis*, an organism which by no stretch of the imagination can be considered a true yeast. True yeast infections may be divided into three groups: (1) Superficial intertriginous cutaneous diseases of an eczematoid character; (2) deep-seated cutaneous or subcutaneous infections tending to become generalized; (3) infections having their origin in the lungs and showing a remarkable tendency to metastasize to the central nervous system. These three clinical types are well differentiated. Excepting the last group, however, the causative organisms are not so easily distinguished.

Superficial Cutaneous Infections.—These infections resemble closely in their distribution and the character of the eruption those produced by *Monilia albicans* and by the Epidermophytons as discussed in preceding chapters. They are found most frequently in the moist folds of the skin, as between the thighs, about the anus, in the folds of the buttocks, under the breasts, in the axillae, and especially between the fingers. That the contact of skin surfaces with the resulting retention of moisture is a prime factor in the etiology is indicated by the report of two cases by Meckel[20] of infections of the hand in which the fingers were held against the palm by the contraction of scar tissue. The condition is apparently fairly common, a considerable number of cases having been reported. Several cases have been associated with diabetes.

The lesions consist of a series of papules, pustules, or blisters, with scaling. The condition between the fingers is referred to as "Erosio interdigitalis blastomycetica." An acneform type has been described by Fabry,[10] a general pemphigus-like eruption by Englehardt.[8] These more severe forms approach cases of the second class of yeast infections. In addition to the above lesions, Ravaut and Rabeau [26] noted in a patient with moist lesions about the vulva and anus, the occurrence of dry, psoriasis-like lesions on the arms and other skin surfaces. Yeasts were isolated from the moist lesions, but not from the dry ones. Injections of an extract of the isolated yeast into the skin called forth dry lesions of the psoriasis type.[27] They suggest that these lesions were due to an allergic reaction similar to the trichophytide observed in ringworms, and designate the lesion "levuride."

In a series of papers published recently by Benedek,[3] a very common skin disease, seborrhoeic dermatitis, is ascribed to a newly described yeast, *Schizosaccharomyces hominis.* Whether this organism bears a causal relationship to the disease is very doubtful. But in any case it is quite apparent from both his descriptions and illustrations that the organism is not a yeast but a bacterium of the *Bacillus megatherium* group.

Deep-Seated Cutaneous Infections.—Probably the first case in which a yeast was definitely proved to be a cause of disease was that reported independently by Busse and by Buschke,[5] observed in 1893. The patient developed ulcers on the face and neck, then a month later swelling of the axillary and cervical lymph nodes and a swelling on the shin-bone. Incision of the latter released a fluid which contained the yeast cells. They were then also found in exudate from the primary ulcers. They were easily cultivated on ordinary media and proved pathogenic for rats and mice. The pathogenicity of the species for man was proved by reinoculation of the patient. The organism was cultivated from the blood shortly before death.

In the exudates the yeast appears almost perfectly round, and in clusters of cells *surrounded by a mucilaginous sheath.* It grew readily on most media, forming a smooth white growth with nothing very characteristic about it on solid media, and a sediment in liquid media. Dextrose was fermented slightly, other sugars not at all. In no media was mycelium formed, and ascospores were never formed.

Mice died in about three weeks after intraperitoneal inoculation with relatively large doses. The organism formed miliary abscesses in the lungs, liver and kidneys. At the point of inoculation there was usually a large mucoid mass composed almost entirely of the yeast cells surrounded by gelatinous material. This gelatinous sheath, however, was not formed in artificial cultures.

Another case was reported by Curtis[7] in 1895. The patient presented two lesions, a large abscess in the right lumbar region and a tumor-like mass in the right groin. The latter was excised and was found to consist largely of gelatinous material, containing the yeasts. The yeast appeared much like that of Busse, save that the cells were apparently somewhat larger, and provided each with a *very thick mucoid capsule.* In cultures the cells were much smaller and without a gelatinous sheath. It gave a very weak fermentation of sucrose, not of other sugars. In general it was not pathogenic for animals, but when injected subcutaneously in rats there developed localized swellings, which were due to a growth of the organism without any inflammatory reaction on the part of the tissues.

Busse claimed to have found one to four ascospores in cultures of Curtis' yeast on plaster blocks. For this reason it is generally referred to the genus Saccharomyces under the name *S. tumefaciens.*

The case of Blanchard, Swartz and Binot [4] was one of intraperitoneal infection. A patient supposed to have tuberculosis of the appendix and peritoneum presented at operation a large gelatinous mass in the lower right quadrant of the abdomen. Microscopic examination showed the same sort of a round yeast with a mucoid capsule. The gelatinous mass was composed mainly of this mucoid material. The yeast grew readily on media, giving the same rather indifferent characters as those of the yeasts cited above. In old cultures the authors claim to have found asci containing eight ascospores. Rabbits, rats and mice were susceptible, but not guinea pigs. Small nodules developed in internal viscera. These contained masses of yeast cells surrounded by the same mucoid gelatinous material, with no trace of inflammatory reaction. The organism has been named *Saccharomyces blanchardi.*

These three cases may be considered typical of a series of probably around twenty or more that have been reported in European literature. Excepting the case of Blanchard and his colleagues they have presented for the most part ulcerating, deep-seated lesions of the skin. Several recently reported from Russia have presented lesions much like those of American "blastomycosis," but with organisms of the Busse-Buschke type. Some cases have yielded to surgical treatment, local applications, and internal medication, but in general they show a tendency to generalize by way of the bloodstream.

These deep-seated cutaneous yeast infections are frequently referred to as "European blastomycosis" to distinguish them from "American blastomycosis" due to *Oidium dermatitidis*. These are also referred to in clinical literature as the "Busse-Buschke" type of blastomycosis.

Torula Infections of the Nervous System.—Some twenty cases, mostly American, have been recorded of a peculiar yeast infection of the brain and meninges. The disease is clinically very distinct. The primary lesions in most cases have been in the lungs, from which focus the organisms have invaded the blood and produced metastatic infections in various viscera, especially the brain, rarely the subcutaneous tissues. Stoddard and Cutler[30] reported two cases in 1916 and reviewed ten others, first clearly distinguishing between this disease and other forms of "blastomycosis." Stone and Sturdivant[31] have very recently summarized nineteen cases. But undoubtedly further cases have been observed that have not been reported.

Most of the reported cases have first consulted a physician for symptoms due to brain involvement, particularly increased intracranial pressure. These cases are very likely to simulate cases of brain tumor, and at least two cases were so diagnosed until autopsy revealed the true condition. The disease develops slowly without fever or other signs of infection.

The lesions consist of areas of destroyed or dissolved tissue containing an abundance of mucoid material and *showing practically no inflammatory reaction.* The complete absence of leucocytes is very striking, though giant cells may be formed. Because of the extensive destruction of the tissues about the parasite, they named the organism *Torula histolytica.*

The parasite appears in the tissues as round, budding cells, surrounded by a thick capsule of the mucoid material which is very evidently secreted by the yeast and not formed by the tissues. These yeast cells may be present in large numbers. In some cases they have been demonstrated in the spinal fluid before death. The capsules may be very clearly demonstrated by suspending some of the sediment from the centrifuged spinal fluid in a drop of India ink and examining the fluid under the coverglass. The yeast cells may show a marked variation in size.

In artificial cultures the morphology is practically identical with that in the tissues save that no capsules are formed. No mycelium is formed. No ascospores have been observed. The organism grows rather slowly, most abundantly on potato and dextrose agar, about as well at room temperature as in the incubator. There is nothing very distinctive about the cultural characters. No gas is formed from any of the usual carbohydrates.

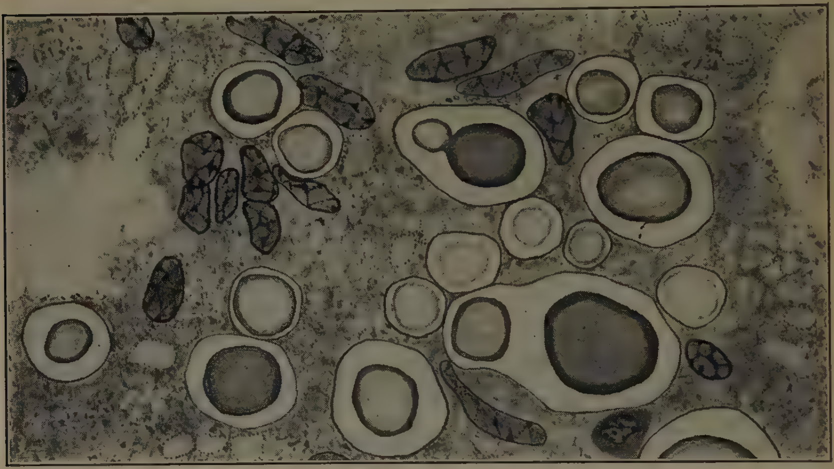

Fig. 90. — Section through the meninges from a case of infection with *Torula histolytica*. Note the variation in size of the yeast cells, and the wide capsular spaces surrounding them. Drawing by Dr. J. C. McKinley.

Rats and mice are particularly susceptible to experimental infection. Inoculations into the peritoneal cavity are followed by a generalized infection with foci in the lungs, liver, spleen and kidneys, as well as the brain. In these lesions there is again an abundant production of gelatinous material with no very marked inflammatory reaction. Epithelioid cells as well as giant cells may occur, forming lesions bearing some resemblance to miliary tubercles.

Classification of Pathogenic Yeasts.—The great majority of the yeasts which have been isolated from various disease processes have been asporogenous. They have been grouped together as a separate genus by Vuillemin under the name Cryptococcus. But, as was pointed out in the preceding chapter, this would seem to be a very poor arrangement because the genus is based upon a single rather uncertain character. Nevertheless, this system has been followed by a number of European authors who have placed in the genus a whole series of yeasts whose sole claim to position there is the fact that they have been isolated from the human body. Undoubtedly a large number of these have had nothing to do with the disease with which they have been associated, being merely accidental contaminants. Also some organisms have been included in this genus by European authors which are not true yeasts, but mycelial organisms like *Oidium dermatitidis* and *Coccidioides immitis.* Thus the generic name Cryptococcus has become quite ambiguous.

The yeasts of Curtis and of Blanchard are supposed to have formed ascospores when first isolated, and are therefore placed in the genus Saccharomyces by most authors. Greenbaum and Klauder[11] found three of four strains isolated from cases of interdigital erosion were spore-forming yeasts. Ota[23, 24] has reinvestigated a number of pathogenic or reputedly pathogenic yeasts, and has found ten strains that form single verrucose spores characteristic of the genus Debaromyces. One of these came from a case of generalized infection of the Busse-Buschke type, two from cases of interdigital erosion, and another from a case of fingernail infection; the remainder were of doubtful origin.

One cannot help being struck by the similarity of the descriptions of the yeasts isolated from most of the cases of infection of the Busse-Buschke type and from the various cases of brain infection reported in this country. In both series of cases the yeasts have appeared in the tissues as quite irregular in form and size, and surrounded by extensive gelatinous capsules; the inflammatory reaction has been slight as compared with the large number of parasites and the extensive destruction of tissue. In cultures, for the most part small round cells without spores are found. The cultural characters are mostly of a negative character. The systematic relationships of the pathogenic yeasts still require considerable study. It will probably be found eventually that there are not nearly so many species as are now recorded in the literature.

Cryptococcus Farciminosus.—A disease of horses very similar to glanders is ascribed to a yeast or yeast-like organism named *Cryptococcus farciminosus.* The disease may begin in a wound or in the mucous membranes of the upper respiratory passages, and spreads by way of the lymph vessels, leading to a chronic progressive disease with both superficial cutaneous ulcers and deep abscesses. The disease has occurred more frequently in Europe and Japan, and was apparently widely disseminated in Europe during the war.

The parasite appears in the lesions as a single-celled organism much smaller than most yeasts (about 2μ in diameter), oval in form, not definitely budding. These organisms occur in enormous numbers within large mononuclear cells, and, as their nuclei stain readily in sections, they appear more like protozoan parasites of the Leishmania type than like yeasts. Some authors have maintained that they are indeed such protozoa. But recent investigators appear to have succeeded in cultivating the organism and to have proved its fungous nature. In cultures, however, it does not grow as a yeast but as a mycelial organism bearing a resemblance to some of the ringworm fungi.

Histoplasmosis.—In this connection mention should be made of a human disease, histoplasmosis, in which there is similarly some question as to whether the parasite is a protozoan of the Leishmania type or a yeast. The disease was discovered in the Panama canal zone by Darling but cases have also been described in the United States. It is rare. It is a generalized disease characterized by fever, leucopenia, anemia, and enlargement of the spleen. The parasite appears much like the organisms just described above, occurring in large numbers in large mononuclear cells of the spleen. It has not been cultivated.

Therapeutic Use of Yeast.—The use of yeast in the treatment of disease goes back to antiquity. Internal consumption is recommended for various skin eruptions, particularly furunculosis, local applications for chronic inflammatory processes, especially leucorrhea. There are not lacking abundant clinical reports on the favorable action of yeast in such conditions, but the therapeutic value of such treatment does not rest upon a scientific foundation. Yeast has no demonstrable bactericidal action. Aside from its vitamin content, it cannot be shown to have any action upon the body other than its action as a laxative.

Bios.—Although yeasts form vitamins abundantly, it is not proved that they require any growth-accessory substances themselves. "Bios" is a hypothetical growth-stimulating substance the existence of which is assumed because with some strains of yeasts the inoculation of "synthetic" media prepared from highly purified chemicals will not result in growth (provided the seeding is very small) whereas growth will take place if minute quantities of various organic extracts are added. There has accumulated considerable literature concerning this substance, which has been reviewed by Tanner.[32] But it would seem that Werkman[37] has settled this problem by continuously cultivating strains of *Saccharomyces cerevisiae* on a medium composed entirely of pure chemicals, synthetically prepared methose being the source of carbon. It is well known that yeasts grow much better with organic sources of nitrogen, and the so-called bios effect is probably due to furnishing such more favorable sources of nitrogen. This view is also supported by Tanner, Devereux and Higgins.[35]

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CHAPTER X

MORPHOLOGY AND CLASSIFICATION OF THE ACTINOMYCETES

No group of microorganisms presents so much difficulty in classification as that now generally designated Actinomycetes. They are mold-like fungi *characterized by extremely fine mycelium* (usually less than one micron in diameter) which is normally nonseptate and either lacks nuclei or possesses nuclei too minute to be resolved by the microscope. They reproduce by conidia formed in chains at the tips of aerial hyphae, and in many cases also by a fragmentation of the mycelium into segments analogous to the oidia of higher fungi.

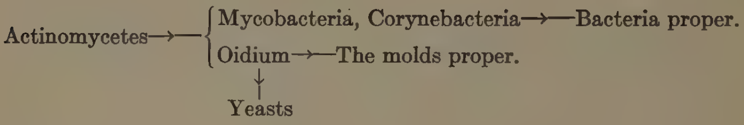
The Actinomycetes are an important group of fungi. A number of species are pathogenic to man and animals. Lumpy jaw in cattle is annually a serious cause of economic loss. A similar disease in man is relatively frequent and has a high mortality. Madura foot is an important disease of the tropics. In addition to these well-known actinomycoses, a large number of other infections due to various species of Actinomyces have been reported. Some of these are caused by a closely related group of acidfast forms which produce a disease very similar to tuberculosis. A disease of potatoes and other root crops, potato scab, is caused by species of Actinomyces and is also responsible for great economic losses. But in addition to these relatively few pathogenic species, there exists a large number of saprophytic forms abundant in the soil which play an important part in the circulation of organic matter in nature. These saprophytic forms are among the most common of the contaminating organisms encountered in bacteriological work.

Systematic Relationships.—In those forms which reproduce by fragmentation of the mycelium, the fragments, or oidia, bear a marked resemblance to rod forms of bacteria. The degree to which this fragmentation occurs varies with the species, or with different cultures of the same organism, but is most frequently encountered in the pathogenic species. In such forms the development of aerial mycelium and conidia may be very slight or completely lacking. On the other hand, when rapidly subcultivated, such forms may fail to develop any mycelium, the growth appearing precisely like a somewhat pleomorphic bacterium, such as the diphtheroid organisms. This observation led early to the view that the Actinomycetes are but higher forms of bacteria. That they are closely related to the bacteria is further indicated by the occurrence of acidfast species of Actinomycetes which bear a close resemblance to the tubercle bacilli, not only in morphology, but also in cultural characters, pathogenicity and immunity reactions. This relationship is so clearly established that there can remain no doubt that the Actinomycetes are phylogenetically very close to the Mycobacteria. Most bacteriologists include the Actinomycetes as a higher group of the Schizomycetes.

Those who have dealt more with the saprophytic strains, on the other hand, have not been so markedly impressed with the similarity to bacteria, since in such forms fragmentation is found less frequently and aerial mycelium with conidia is produced more regularly. They have been more prone to group them with the higher molds, as the Fungi Imperfecti, or to give them rank as a separate class between the Schizomycetes and the Fungi Imperfecti. Thus Vuillemin, in his classification of the Fungi Imperfecti, first included the Actinomycetes as a separate order under the name Microsiphonales, but later gave them the rank of a family (Nocardiaceae) in his order of Arthrosporales, giving more weight to the fragmentation of the mycelium as a character of taxonomic importance. Drechsler [4] could see no resemblance to bacteria and would include them without qualification as a group of the Fungi Imperfecti. Lieske [5] prefers to consider them an independent group of fungi, between the molds and the bacteria, but showing a more intimate relationship with the bacteria than with the higher fungi. Waksman[10] believes that "they should be looked upon as a group of fungi, to be classified separately from other groups till their exact systematic position has been definitely established."

Petruschky [7] proposed some years ago an arrangement which is still followed by some textbooks of bacteriology. He made a separate class of fungi characterized by the formation of very fine filaments which he designated Trichomycetes. This included four genera: Actinomyces, including forms with true branching and forming "clubs" in infected tissues; Streptothrix, with true branching but not forming "clubs"; Cladothrix, with false branching; and Leptothrix, with no branching. But as will be pointed out later, the formation of "clubs" in the tissues is not a reliable character and certainly not sufficiently specific to be used for generic differentiation. And Cladothrix and Leptothrix are ensheathed iron bacteria bearing no similarity to the Actinomycetes but rather closely related to the blue-green algae. There is, therefore, no good reason for continuing Petruschky's classification.

Concerning the origin and evolution of the Actinomycetes, three possibilities have been considered: (1) that they are a higher development of the bacteria; (2) that they are degraded molds; and (3) that they represent a common ancestral type from which both bacteria and molds have developed. We hardly know enough about them yet to warrant such speculation, but all three theories have had their supporters. Lieske [5] leans towards the third viewpoint because of the fact that the Actinomycetes are very labile and prone to vary in their morphology, at times towards the bacteria, at other times towards the molds. The following scheme taken from his book illustrates the suggested relationship to the bacteria and to the higher fungi:



Lieske places organisms of the type of *Oidium lactis* very close to the Actinomycetes because of the fragmentation of the mycelium common to both groups, an opinion also held by Vuillemin.

Since the "oidia" or "fragmentation-spores" of Actinomycetes resemble bacteria so closely, and since some species of Actinomycetes may at least temporarily fail to form mycelium and grow entirely in a bacteria-like form, one might rationally assume that bacteria bear the same relationship to Actinomycetes as yeasts bear to larger molds, i.e., that they represent Actinomycetes which have permanently lost the power to form mycelium. This may be considered as very probably true for the acid-fast bacteria and the diphtheroid group. But it remains to be proved that the bacteria are themselves phylogenetically a homogeneous group, and therefore it would be unwise to assume a relationship of all bacteria to the Actinomycetes.

Synonymy.—It is very difficult to read the literature on this group of microorganisms intelligently because of the multiplicity of names which have been applied, sometimes to the group as a whole, sometimes to portions of it. The following are used as synonymous with Actinomyces: Streptothrix, Cladothrix, Oöspora, Nocardia, Discomyces.

The name Streptothrix was applied by Cohn to the first species of the group to be discovered, *Streptothrix foersteri*. This name was, however, previously used to designate one of the higher molds and is, therefore, not eligible. It has, however, been used by Foulerton, and by Musgrave, Clegg and Polk, to designate the entire genus, and, as indicated above, by numerous bacteriologists, following Petruschky, to designate those pathogenic forms not producing "clubs" in tissues.

The name Cladothrix was first applied to a group of the iron bacteria of which *Cladothrix dichotoma* is the type species. This is an organism composed of chains of cells in a sheath, showing "false" branching. No one acquainted with this organism would consider it closely related to the Actinomycetes. Nevertheless, Eppinger named the acidfast Actinomyces which he discovered, *Cladothrix asteroides*, under the mistaken impression that it exhibited false branching, and this name has been handed down through the literature.

The name Oöspora has been applied to some members of the group by certain French bacteriologists, and Thaxter named the parasite of potato scab *Oöspora scabies*. The genus Oöspora is rather poorly defined, but is usually considered to include certain of the higher molds with septate mycelium and definite nuclei, and therefore the Actinomycetes do not belong here. Nevertheless this name is preferred by Sartory.

The name Nocardia, given by Trevisan to the group to honor Nocard who discovered one of the important species, has been used by some French authorities to designate the whole group, by some writers to designate the saprophytic species. It has not come into general use and must be ruled out by the laws of priority.

Apparently Rivolta observed the organism of actinomycosis in cattle before it was accurately described by Harz, and named it

Discomyces bovis. This term Discomyces has therefore been used by some French writers.

The name Actinomyces was given to the organism of lumpy jaw in cattle by Harz. It has the advantage over all of the other terms used in that it fulfills the law of priority, being the first name applied to a member of the group accurately described which had not been used for some other fungus;* in being descriptive; and in that it is more widely used than the others. Fortunately the synonymy of the various terms is coming to be generally recognized, as well as the validity of the term Actinomyces. The name means "ray fungus," and these organisms are frequently referred to in English literature as ray fungi, and in German literature as "Strahlenpilzen."

The very involved question of synonomy and terminology of the Actinomycetes has been thoroughly discussed by Breed and Conn.[1]

Structure of the Mycelium.—The Actinomycetes are characterized by the extreme fineness of their mycelium. Rarely more than 1.5μ in diameter, it is frequently less than half that in thickness. The mycelium is, then, no thicker than, say, a typhoid bacillus, and usually reveals no more internal structure than does that bacterium.

This mycelium is branched, and in some species rather twisted and curled. It forms a tangled mass like the mycelium of any of the higher molds. Although the branching has been described as dichotomous by some authors, this is not the case. There is always a main or axial filament with lateral branches.

In most Actinomycetes examined the mycelium appears to be perfectly homogeneous, i.e., undifferentiated, whether examined unstained or in fixed and stained preparations. But in older parts of the mycelium it frequently becomes thicker and one can distinguish within it granules and vacuoles. In certain forms, especially in old cultures on rich media, these vacuoles may become quite numerous and large.

Nuclei.—In preparations stained by certain methods, deeply stained granules may sometimes be demonstrated. There has been some discussion as to whether or not these are to be considered nuclei. They may be completely absent in the younger mycelium, may first appear as small isolated granules, and may occur as numerous large masses in the older mycelium. Most authors are inclined to interpret them as volutin, but no critical researches based upon microchemical reactions have been published. Drechsler,[4] on the other hand, believes that nuclei may be demonstrated in the developing conidia.

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* The application of the name "Actinomyce" for a mistakenly identified Basidiomycete by Meyen in 1827 can hardly be seriously considered as invalidating the use of "Actinomyces" for the ray fungi on the grounds of priority.

Septation of Mycelium.—There has also been some difference of opinion concerning the occurrence of septa in the mycelium. Drechsler states that septa are present, but that the cells so formed are very long. Most other investigators deny the existence of septa. It must be admitted that with such fine mycelium it is rather difficult to determine whether certain rather infrequent interruptions in the continuity of the mycelium are septa or vacuoles.

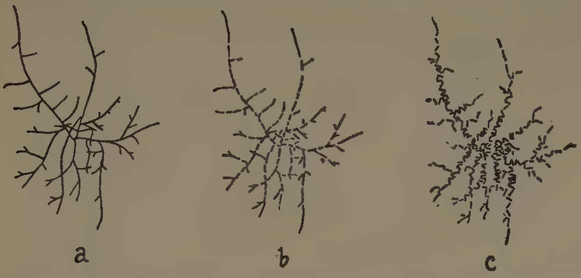

FIG. 91.—Diagram showing origin of bacterial forms from mycelium in Actinomycetes. a. Continuous mycelium. b. Fragmentation. c. Postfission movements lead to "angular growth."

Fragmentation of Mycelium.—The frequent occurrence of fragmentation of the mycelium into segments analogous to the oidia of organisms like *Oidium lactis* has been considered as evidence of the occurrence of septa in the mycelium. It may well be, however, that this fragmentation occurs in the same manner as the fission of bacteria, i.e., that no cross-wall is formed at all or else that it develops just preceding the division. This formation of oidia occurs very early and regularly with some species, and not at all with others, and has been proposed as a basis for a major subdivision of the group by Orskov.[6] In general those species which tend to form pellicles on liquid media tend to undergo mycelial fragmentation, and those that grow in the bottom of the tube do not, but this is by no means an absolute rule.

The formation of oidia or fragmentation spores has been studied particularly by Orskov.[6] The fragments frequently occur in a characteristic zigzag arrangement, which is best seen in slide cultures. According to Orskov, fragmentation begins at the center of the colony and proceeds peripherally. After the mycelium has split into elements of fairly uniform length, these undergo post-fission movements of precisely the same character as those shown by the diphtheroid bacteria, one element moving through an arc to lie at an angle with the adjacent element. This Orskov refers to as "angular growth." It is particularly characteristic of the pathogenic species, and may be considered as further evidence of a relationship to bacteria. Such a zigzag arrangement of the elements produced by fragmentation of the mycelium is also characteristic of *Oidium lactis*.

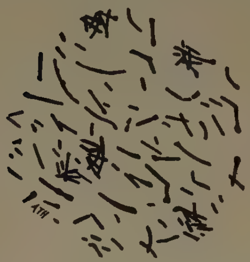

FIG. 92.—Smear preparation from a culture of *Actinomyces bovis* (Israel type), showing bacterial forms resulting from fragmentation of mycelium. When rapidly subcultured the growth becomes purely bacteria-like, no mycelium developing. Note the resemblance to diphtherioid bacteria.

When smear preparations are made from fragmented cultures of Actinomycetes, naturally the arrangement of the mycelial fragments is disturbed, they become irregularly scattered. Here and there will be found two cells in a V-shaped arrangement. The appearance of the slide is precisely that of a rather pleomorphic bacterium, such as the diphtheroid organisms. If in addition some conidia have been formed, these will appear very much like cocci in chains, and the resemblance to a smear preparation of mixed bacteria is very close indeed.

Conidia.—In addition to the oidia or "fragmentation spores," many species of Actinomycetes reproduce by the formation of conidia on aerial hyphae. Spore formation is, however, very irregular, with many species failing completely on some media. Cultures which have not been transplanted for some time frequently fail to form spores in the first subculture. It is not an uncommon experience to find conidia rather suddenly develop on a strain which has not shown any aerial mycelium during many months of subcultivation. The formation of aerial mycelium and conidia is manifested by the development of a fine powdery coat on the surface of the culture.

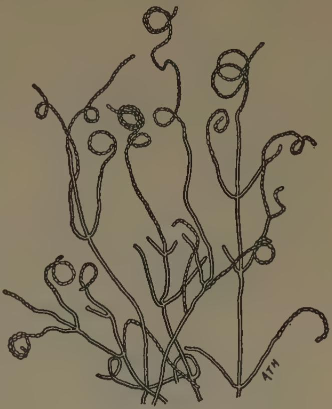

Fig. 93.—Conidia and conidiophores of *Actinomyces violaceus-ruber*. Drawn from a slide culture. Note that both dextrorse and sinistrorse spirals are found in the same preparation.

The mechanism of spore formation and the arrangements of the sporogenous hyphae have been studied in some detail by Drechsler.[4] According to this author, conidia are formed by the development of septa in the terminal portions of the filaments. These septa are at first thick and stain deeply. Then a division occurs transversely through the septa separating the terminal portion of the filament into cylindrical segments which later may become rounded. But Lieske[5] believes that true septa are not formed. Instead, vacuoles or empty spaces appear in the hyphae, dividing the protoplasm into so many segments which become separated by constriction to form the spores. According to this author, the aerial spores are not to be considered conidia, but merely a further development of "fragmentation spores." It would seem, however, that the aerial mycelium and its spores are too minute to permit of such fine differentiations to be made with certainty.

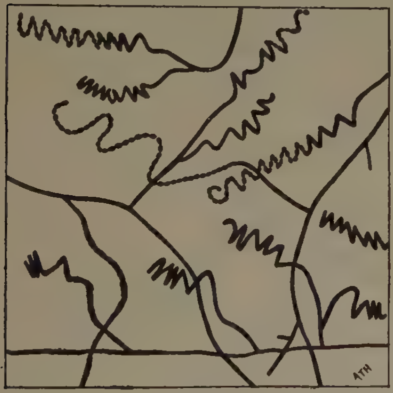

Fig. 94.—Conida and conidiophores of *Actinomyces viridochromogenus*.

Conidiophores.—A very striking character of the spore-bearing filaments is their tendency to be spirally twisted. This is more marked in some species than in others. Drechsler has noted that in some cases the filaments are coiled to the right, in other cases to the left, and that this character is constant for the species.

The spiral curvature begins to develop as the spores are formed and increases during their development. When the spores are mature, the filaments untwist and this mechanism may serve to help discharge them into the air.

The spore-bearing aerial hyphae are branched, and this branching may also be characteristic of the different species and serve as a means of differentiation. Drechsler recognizes two main types: (1) "an erect dendroidic type in which the sequence of development of the sporogenous hyphae is successive; and (2) a prostrate, racemose type in which the development is more nearly simultaneous." In addition, Waksman[10] has described a type in which the spore-bearing branches are given off from the main or axial filament in whorls. It remains to be demonstrated, however, that these characters of the conidia and conidiophores are sufficiently constant to serve as diagnostic characters.

Clubs.—In the infected tissues in the "lumpy-jaw" type of Actinomycosis in cattle and in the similar disease in man, as well as in those cases of Madura foot due to Actinomycetes, the fungus has a very characteristic morphology not usually exhibited in cultures. The organisms appear as small granules which on section show a structure such as is illustrated in Fig. 95. There is a central mass of closely intertwined fine filaments of mycelium, and a peripheral lobulated structure. The central mycelium stains with the basic dyes, the peripheral portion takes the acid dyes. This peripheral lobulated structure is frequently lacking at one side, from which strands of fine mycelium trail off into the tissues.

The peripheral lobulated structure is made of "clubs," i.e., characteristic swollen tips of the filaments of mycelium. In favorable sections when examined by very high magnifications they are seen to consist of a cap or sheath of the hyaline acid-staining material fitting over the tip of the filament of mycelium.

They have been the object of considerable study and speculation. There are several theories as to their nature and function. They have been considered to be spores, but this is evidently not the case, as there is no evidence that they ever separate from the mass and germinate. It has been suggested that they are organs of absorption similar to the bulbous extremities of the mycelial filaments of some plant parasites which penetrate the cells of the host plant. Some authors have held that they are a product of the body tissues rather than of the parasite, serving to wall it off from the rest of the body. Microscopic observation, however, clearly indicates that these structures are developed from the fungus, and in rare cases, as has been described by Bayne-Jones, they may be formed in artificial culture media. The most likely theory is that they represent a secretion of the organism or a modification of its cell wall brought about by the action of the body fluids upon it.

It is clear that the formation of these "clubs" depends upon a certain balance between the virulence of the organism and the resistance of the invaded tissues, a balance which occurs most frequently with the species named. Other pathogenic species are more virulent. But if their virulence is attenuated, as has been done with *Actinomyces asteroides*, and they are then inoculated into animals, the clubs will develop. Moreover these structures are not entirely peculiar to the Actinomycetes. Very similar lobulated masses of acid-staining hyaline material have been observed about clusters of attenuated tubercle bacilli injected into animals, as well as about clumps of the normally non-pathogenic acidfast butter bacilli inoculated in massive doses, and also surrounding masses

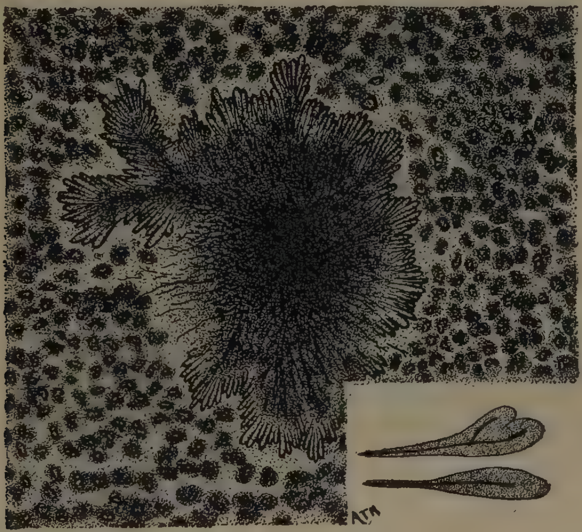

Fig. 95.—Section through an actinomycotic abscess, showing the structure of the actinomycotic granule. Insert, clubs highly magnified (semi-diagrammatic).

of attenuated Staphylococci in the disease of horses known as "botryomycosis," as well as in certain cases of Aspergillosis.

Therefore, although these structures are very constant and of great diagnostic value in the actinomycoses in which they occur, they cannot be considered as highly specific structures, and particularly should not be used as a basis for generic differentiation as has been done by Petruschky and others.

Cultural Characters of the Actinomycetes.—The colonies of the Actinomycetes must be familiar to all who have ever exposed agar plates to the air, either intentionally or accidentally, for their spores are ubiquitous. Small, round, flat, tenaciously adherent to the medium, the surface covered with a white or grayish powdery film, the surrounding medium often dark brown in color, and very frequently accompanied by a most penetrating musty odor, they are easily recognized.

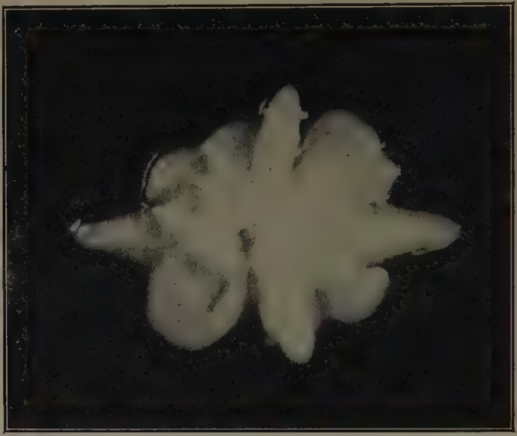

Fig. 96.—Colony of a bacterium-like Actinomyces (*A. bovis*, Israel type).

Colonies.—Older colonies vary considerably in appearance. In general two main types may be recognized, corresponding to the forms that do and do not readily undergo fragmentation of the mycelium. The first type forms colonies which are very firm, almost cartilaginous in consistency, and very adherent to the agar. They are usually slightly conical in cross-section, frequently have an elevated central papilla, and show marked radial foldings. Such forms are at first smooth and glistening on the surface, but later develop spores, when the surface develops the powdery appearance mentioned above. Not infrequently the aerial hyphae do not develop all over the surface, but in concentric rings. In the second type the colonies are not so tenacious, but tend rather to be of a mealy consistency, and are not so adherent to the agar. Their surface is usually irregularly wrinkled in all directions, not showing the radial folds. These colony forms are not perfectly constant and species may be found which will form one type at one time or on one medium, and the other type at another time or on a different medium.

The growth on agar slants in general varies as does the character of the colonies.

Growth in Liquids.—On liquid media also, two general types of growth may be recognized, which again correlate roughly with the types of colonies described above. The non-fragmenting, tenacious colony types usually grow submerged in the liquid, and form small fluffy masses of mycelium either at the bottom of the tube or adherent to the sides. The fragmenting, mealy colony types tend to form a dry wrinkled pellicle floating on the surface. But again these characters are not altogether constant, and the correlation of these characters is by no means perfect.

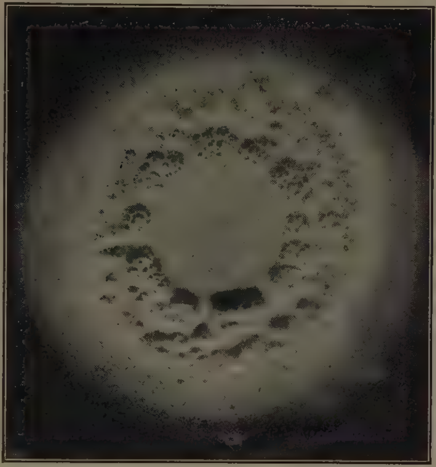

Fig. 97.—Colony of a mold-like Actinomyces (*A. griseolus*).

Pigments.—One of the most striking and important of the cultural characters of the Actinomycetes is their production of pigments. Three types may be recognized: that which develops in the spores, that which is retained in the mycelium, and that which diffuses into the medium.

The spores are usually white or gray, sometimes a distinct brownish-gray or olive-gray.

The mycelium may be nearly colorless, having a rather indefinite yellowish translucent appearance, or it may be brilliantly pigmented. Practically all of the colors of the rainbow may be found if a number of species are studied; blue, green, yellow, orange and red.

The "Tyrosinase Reaction."—A considerable number of species produce a very characteristic brownish discoloration of the agar. This color is formed usually only on media containing protein, not on "synthetic" media. It is very pronounced on potato and gelatine, and easily noticeable in the water-clear peptone solution. At first yellowish, it becomes gradually light and then dark brown, and finally in some cases almost black.

The formation of this pigment, according to Beijerinck, is due to the action of an enzyme, tyrosinase, on tyrosin, converting this substance to a pigment, melanin; this reaction is supposed to be the same one which causes the spontaneous darkening of potatoes when their cut surface is exposed to the air. But according to Waksman,[8] many species will not form this brown pigment in media containing pure tyrosin as the source of nitrogen, which will form the pigment in media containing proteins; while the brown pigment may be formed in proteins, such as gelatine, which do not contain the amino-acid tyrosine. It is, therefore, doubtful if the tyrosinase reaction is the cause of the brown pigment. It should also be noted that occasional strains may bring about a brownish discoloration in synthetic media containing no protein; but these exceptions may be due to a reaction with some protein substance synthesized by the organism itself.

It was formerly believed that the brownish discoloration of the media was characteristic of a single species named *Actinomyces chromogenus*, but it is now known that a considerable number of strains which differ among themselves in other characters may form this brown pigment. These forms are frequently referred to as the "chromogenus group" of Actinomycetes, and individual species may be given compound names, such as *A. viridochromogenus*, indicating a variety which forms a brown discoloration of the agar and a green pigment in the mycelium.

In addition to the brownish discoloration of the medium common to a number of species of Actinomycetes, many varieties form truly specific soluble pigments. These are usually of the same color as the pigment retained in the mycelium, but may be different. Yellows and oranges are most frequently observed.

Variability of Colors.—The pigmentations are subject to extreme variation. In general, pigmentation is most pronounced in strains which have been recently isolated from their natural habitat, especially with soil strains, and tends to disappear more or less rapidly upon continued cultivation on artificial media, especially if frequently transferred. The color may vary greatly with the composition of the medium and with the age of the culture. One species, *Actinomyces violaceus-ruber*, regularly shows a very striking color change, being at first red, later a pronounced blue. This has been shown to be due to a change in the reaction of the medium, the pigment being very similar to litmus, and changing from red to blue as the reaction of the medium changes from acid to alkaline. This pigment also diffuses into the medium. Probably in other cases where the organisms change color with changes in the composition of the medium it will be found that this is due to variations in the reactions of the medium, the pigment acting as an indicator.

Although pigment production may vary greatly with even relatively slight changes in the composition of the medium, it may be kept reasonably constant if the medium is one of precise composition which can be readily duplicated. For such a purpose, as was described in Chapter II, a "synthetic" medium composed of pure chemicals is especially desirable. As has been pointed out by Waksman,[8] pigment production is more constant in media of rather low nutrient value, and also much more striking. Many species, which form no pigment on a rich medium like dextrose meat extract agar, may give brilliant pigments on a medium with an inorganic source of nitrogen and a sugar which is not so readily utilized as a source of carbon. Such a medium is Czapek's agar, containing sodium nitrate and sucrose. Pigment production of the Actinomycetes in this medium has been particularly studied by Waksman, and there is no question but that on such a medium pigment production is sufficiently constant and striking to be a very valuable aid in identifying the various species. particularly the soil forms.

Conditions of Growth.—Most of the Actinomycetes grow well on all of the usual bacteriological culture media. Some of the pathogenic strains grow very poorly, and some have never been cultivated, but these are exceptions. For the most part they prefer an alkaline medium, and many species are sharply inhibited by relatively slight degrees of acidity. For most soil species the limiting hydrogen-ion concentration is pH 5.0. With most strains there is a tendency for the medium to become more alkaline during their growth. They vary somewhat in their temperature requirements, but most varieties will grow almost equally well at room temperature and in the incubator. A few soil forms are distinctly thermophilic, growing readily at 60°. Such forms have been isolated from heating hay. In general their upper limits are the same as for bacteria, about 42° C. The thermal death point of the mycelium is from 60 to 70°, of the spores about 5° higher. Most forms are strictly aerobic, but some of the pathogenic forms are more or less anaerobic, at least when first isolated. These will be discussed later.

Biochemical Activities.—The biochemical activities of the Actinomycetes have been rather extensively investigated by Waksman,[9] whose papers should be consulted for detailed information. A wide variety of organic substrates may be utilized by this group of microorganisms. The simple sugars are readily utilized without fermentation, the media generally becoming alkaline in reaction. Sucrose is rarely inverted and is not a good source of carbon. Nearly all are actively diastatic, breaking up starch with great rapidity. Some species also split cellulose. Proteins are digested by most species, gelatine, casein and blood serum being liquefied. Egg albumen, on the other hand, is not digested by most varieties. The breaking down of proteins to amino acids proceeds rapidly, the conversion of the latter to ammonia takes place much more slowly, and may completely fail with some species. Nitrates are more readily utilized than ammonium salts, probably because the utilization of the former makes the medium more alkaline, the latter more acid. The majority of species can reduce nitrates to nitrites, but not to ammonia or free nitrogen. Some species are haemolytic. A number of forms produce a rennin coagulation of milk. Fat-splitting enzymes may be demonstrated in a number of species, and Actinomycetes have been found to grow in butter with a definite production of acid. Actinomycetes have been found growing on rubber and decomposing it.

Classification of the Actinomycetes.—There are undoubtedly a great many distinct species of Actinomycetes. Waksman states that more than fifty species have been isolated from soil. Brumpt lists some seventy species as having been isolated from lesions of man and animals; Sartory includes ninety-odd, named or not. Lieske lists seventy-six species. But in many cases these species have been very inadequately described, and in some cases new species have been created without good reason, so that undoubtedly a number of synonyms are included in these lists.

The extreme variability of the Actinomycetes in both morphological and cultural characters offers a serious obstacle to all attempts to classify and identify them. This variability is perhaps not so serious as some authors would make it out, as it can be controlled to a certain extent by the use of standardized media of definite composition, as was pointed out above. Nevertheless it is largely due to this variability that our knowledge of the species of Actinomycetes is so uncertain and more or less chaotic.

A personal experience may serve to illustrate the difficulties involved due to this variability. The author, with Gardner, has described a species of acidfast Actinomyces under the name *A. gypsoides*. When first isolated, it differed from the previously described species in being actively proteolytic, peptonizing milk, liquefying gelatine, and producing a pronounced brownish discoloration of protein-containing media. These characters were retained for some months. But it gradually lost its proteolytic powers, first failing to peptonize milk, and then to liquefy gelatine. The latter property was revived temporarily by growth in a medium containing no source of nitrogen save gelatine, but was eventually completely lost. At the present time subcultures of this organism present no characters by which it may be differentiated from *A. asteroides* save the definite brown color of the agar and a more pronounced tendency to form aerial mycelium. Many old laboratory strains of Actinomycetes similarly fail to fit their original description.

It is only within relatively recent years that the large number of saprophytic forms of Actinomycetes has been known. Only a relatively small number of investigators have made any extensive study of the group. Authors who have been interested in the pathogenic forms have not paid much attention to the saprophytic forms, and vice versa.

For these various reasons any classification which may be presented at the present time must be considered as more or less tentative and any list or key to species must be considered as being only suggestive and not at all perfect.

Various characters have been proposed by different authors as a basis for a subdivision of the group into subgroups, either genera or families as the case may be, or leaving the rank of the subgroups undetermined. Thus, as has been mentioned, Petruschky divided the branched filamentous organisms into two groups, Actinomyces and Streptothrix, on the basis of club formation.

Drechsler[4] believes that the type of branching of the conidiophores, the degree and direction of their coiling, and the mode of septation in the process of spore formation may be used as differential characters. But Waksman has found these characters rather variable with different media. Such characters would not be of value with many of the pathogenic forms which do not produce conidiophores.

Waksman[10] proposes the following tentative subdivision based upon the characters of the sporogenous hyphae:

1. Presence of substrate mycelium alone, no aerial mycelium formed on organic or synthetic media.

2. The aerial mycelium consists of very long filaments, rarely branching and mostly sporogenous almost to the point of origin in the nutritive mycelium, without any coiling. This group can be subdivided into two groups: (*a*) long mycelium and little branching, (*b*) short mycelium and abundant branching.

3. Maturation of the sporogenous hyphae associated with the formation of characteristic spirals. A flexuous habit of the young filament early manifesting the tendency towards the coiling condition which becomes more definite with continued elongation. This group can also be divided into subgroups on the basis of the obliquity of the spiral, diameter of the turns, construction with reference to the dextrorse and sinistrorse condition (constant characters, according to Drechsler). The spirals range from open, barely perceptible turns to strongly compressed spirals, so that the adjacent turns are in continuous contact. The number of turns ranges from two or three to twenty or more.

4. Sporogenous hyphae formed in knot-like groups of three or four along a central hypha.

Orskov[6] has also proposed a subdivision of the group upon morphological characters, recognizing three main subgroups:

1. Sporogenic forms. The spores develop into a unicellular substrate mycelium that does not divide spontaneously. Out of this mycelium arises an aerial mycelium which later divides by the

breaking up of the protoplasm into regularly sized parts. These are separated from one another by constriction of the thread membrane between the individual elements. They grow in the form of flakes in the liquid media, usually at the bottom. The name Cohnistreptothrix was suggested for this group.

2. An initially undivided substrate mycelium is formed with an early development of aerial hyphae. Both substrate and aerial mycelia divide spontaneously into segments. There is early surface growth on liquid media. It is suggested to reserve the name Actinomyces for this group.

3. A unicellular delicate branching mycelium, at the extreme tips of which single oval spores are formed. There is growth in liquid media in the form of small firm grains at the bottom and along the glass. The name Micromonospora is suggested for this group.

The third group is extremely rare, the great majority of strains falling in the first two. It will be seen then that the major subdivision in Orskov's arrangement is based upon the presence or absence of fragmentation, a character which is definitely correlated with the character of growth on both solid and liquid media, as was pointed out above. These characters seem to be fairly constant.

The following arrangement of the Actinomycetes, taken from Brumpt,[2] is based upon the ideas and nomenclature of Vuillemin, Pinoy, Foulerton and Langeron. This classification is followed also by Castellani [3] and in part by Sartory:

Microsiphonales

Hyphomycetes with very fine mycelial filaments, not segmented, in diameter one micron or less, often laterally branched, with homogeneous contents, without distinct nuclei, often dissociating into bacteroid or coccoid fragments.

A. Difficult to cultivate, generally anaerobic, at times also aerobic; not producing spores in cultures. *COHNISTREPTOTHRIX.*

(Note that this definition of Cohnistreptothrix is quite different from that of Orskov, who applies the name to aerobic, non-fragmenting, conidia-forming types. This term was introduced by Pinoy to differentiate the Israel type of Actinomyces (see page 263) from the aerobic species.)

B. Generally aerobic, but some can grow anaerobically. Forming spores. *ACTINOMYCES.*

1. Species easily cultivated, producing on solid media a white, chalky efflorescence. Filaments branched, more voluminous than in the other

groups, in general not acidfast. Cultures give off a musty odor. Grow easily on potato, also on gelatine and coagulated serum, the latter two being liquefied. Starch is digested. Section *MAJORA*.

(This section would include the Bostroem type of Actinomyces (see page 262) as well as the majority of saprophytic species.)

2. Easily cultivated, on solid media the cultures are dull, finally powdery, generally without odor, resembling those of the tubercle bacillus. Filaments rarely branched, almost always acidfast. Not liquefying gelatine or blood serum, or only one of the two. Growth on potato poor. These species are the most pathogenic, often causing generalized infections. Section *MINORA*.

(The acid-fast *Actinomyces asteroides* and related species, and the organism of Madura foot, *Actinomyces madurae*, fall in this section. It corresponds fairly well with Orskov's second group.)

3. Cultivated with difficulty. The most favorable medium is peptone bouillon, in which they form small isolated colonies adherent to the bottom or walls of the tube. On solid media no efflorescence is formed. Filaments are not branched, reduced to short bacilliform fragments. Neither gelatin nor blood serum are liquefied, not growing on potato, and not producing diastase. Section *BREVIORA*.

(This section contains a number of forms not well known, obtained from various types of infection.)

Castellani [3] has published a scheme for identification of a considerable number of species based upon the above classification.

Species of Actinomycetes.—In the present state of our knowledge it is not easy to identify species of Actinomycetes. This is particularly true of the pathogenic species. Undoubtedly some of the described species are identical with others previously described, and some are not Actinomycetes, but diphtheroid types of bacteria. The final identification must be based very largely upon cultural characters, and more particularly upon pigmentation. This is especially true of the saprophytic soil forms which have been so intensively studied by Waksman. Waksman has particularly emphasized the necessity for using synthetic media of precise composition. A list of his "routine" media has been presented on page 37.

WAKSMAN'S KEY TO THE IDENTIFICATION OF SPECIES OF SOIL ACTINOMYCETES[10]

(Based chiefly on physiological characteristics)

A. Formation of a soluble pigment on all media containing protein substances:

- I. Pigment deep brown (Chromogenus types):
  - 1. A brown pigment is produced on tyrosine agar:
    - a. Pigment dark brown; white to cream-colored growth on synthetic media; soluble brown pigment on synthetic media containing arabinose, glucose or lactose. *A. scabies.*

      A number of strains of Actinomyces were isolated from potato scab lesions; it was suggested that there is no justification for including all these organisms in one species. (Millard and Burr.)
    - b. Pigment faint brown; sulfur-yellow soluble pigment on creatinine solution; aerial mycelium on glucose agar is ochre to reddish-ochre-colored. *A. olivochromogenus.*
  - 2. Growth and aerial mycelium on synthetic agar green to dark green; soluble brown pigment on synthetic media with most carbohydrates. *A. viridochromogenus.*
  - 3. Deep brown to black pigment on synthetic agar:
    - a. Weakly growing organisms; orange-red growth on potato plug; no visible aerial mycelium on synthetic agar. *A. purpeochromogenus.*
    - b. Vigorously growing organisms; brown to black growth on potato plug; abundant cottony aerial mycelium on synthetic agar. *A. pheochromogenus.*
  - 4. Usually no action on milk (37°), accompanied by the darkening of the milk; mouse-gray aerial mycelium on synthetic agar; ammonium salts used readily with different sources of carbon. *A. aureus.*
  - 5. Brown pigment never produced on synthetic media:
    - a. Aerial mycelium on synthetic media has lavender shade. *A. lavendulae.*
    - b. Aerial mycelium on synthetic agar is abundant, of a water-green color. *Actinomyces* 218.
    - c. Whorl formation in aerial mycelium on synthetic agar:
      - a'. Growth colorless and aerial mycelium white. *A. reticuli.*
      - b'. Growth pink, aerial mycelium rose-colored; nitrate reduction very abundant; fewer whorls. *A. reticulis-ruber.*
    - d. Growth on synthetic agar sulfur-yellow, with yellow aerial mycelium; barnacle-like, greenish-yellow growth on potato plug. *A. flavus.*

e. Growth on synthetic agar red-colored, aerial mycelium abundant, orange-colored; aerial hyphae usually do not form spirals. *A. ruber.*

II. Soluble pigment on organic media faint brown, golden, yellow or blue:

1. Pigment blue, not always definite; soluble red pigment turning blue on synthetic agar. *A. violaceus-ruber.*

2. Pigment at first green on organic media and synthetic agar, property lost on continued cultivation, becoming brown on synthetic agar; aerial mycelium not produced on most media. *A. verne.*

3. Soluble pigment at first brown, property lost entirely on continued cultivation; growth and aerial mycelium on synthetic agar abundant, white. *A. albus.*

4. Soluble pigment yellowish green; growth on synthetic agar penetrating into the medium is pink. *A. californicus.*

5. Brown pigment produced only on certain protein media (usually gelatine and glucose broth, not nutrient agar):

a. Growth on synthetic agar red to pink; no differentiated aerial mycelium or only scant white. *A. bobili.*

b. Growth on synthetic agar colorless; aerial mycelium thin, rose-colored. *A. roseus.*

c. Growth on carrot and potato rapidly spreading, enveloping the whole plug and destroying it rapidly, plug becoming colored deeply brown. *A. griseolus.*

d. Red (Vinaceous) soluble pigment on synthetic agar, often turning red-brown; white aerial mycelium. *A. erythreus.*

B. No soluble pigment produced on gelatine or other protein media:

I. Species strongly proteolytic; gelatine liquefied rapidly, milk clotted and peptonized rapidly.

1. Brown soluble pigment on synthetic agar; diastatic action very strong. *A. diastaticus.*

2. Rapid liquefaction of coagulated blood serum, strong hemolysis of blood (37°); aerial mycelium on synthetic agar has a tea-green tinge. *A. griseus.*

3. Yellowish-green growth on starch plate with pinkish aerial mycelium; citron-yellow growth on synthetic agar. *A. citreus.*

4. Greenish-yellow growth on synthetic agar, gray powdery aerial mycelium, greenish-yellow soluble pigment. *A. flavovirens.*

5. Colorless growth on synthetic agar, white to grayish aerial mycelium, no spiral formation; thin reddish-brown growth on potato plug (purplish zone on plug); faint yellow pigment may develop on gelatine, blood and egg-media. *A. poolensis.*

6. Buff-colored growth on glucose agar, violet-gray aerial mycelium; yellow growth on synthetic agar with light drab aerial mycelium; rapid destruction of potato plug. *A. olivaceus.*
7. Very scant colorless growth with scant white aerial mycelium on synthetic agar and on synthetic media containing $NaNO_3$ as a source of nitrogen; abundant brown growth with white aerial mycelium and soluble brown pigment on glucose agar; growth on potato plug greenish, turning black. *A. gelaticus.*

II. Proteolytic action weak:

1. Soluble pigment produced on synthetic agar:
    a. Pigment blue or blue-black. *A. violaceus-caesari.*
    b. Pigment brown to almost black on all synthetic media with $NaNO_3$ as a source of nitrogen. *A. exfoliatus.*
2. No soluble pigment on synthetic agar, although growth is colored:
    a. Growth turning black, diastatic action very strong:
        a′. Growth on synthetic agar scant with abundant spirals in aerial mycelium, no invertase production. *A. rutgersensis.*
        b′. No spirals on synthetic agar, characteristic green-colored growth on protein-glycerine media. *A. lipmanii.*
        c′. None or scant aerial mycelium on all media; growth abundant on synthetic agar (invertase positive); none or scant growth on blood agar and egg-media. *A. halstedii.*
    b. Growth orange-colored on most synthetic and organic media; aerial mycelium pink. *A. fradii.*
    c. Growth yellowish on synthetic and glucose agars; pinkish to cinnamon-colored on calcium malate agar; no growth on blood serum and egg media; none or only very scant and late aerial mycelium on most media. *A. alboflavus.*
    d. Growth on synthetic media rose to red-colored, aerial mycelium white, no visible action on milk. *A. albosporeus.*

Complete descriptions of the above species may be found in Waksman's "Cultural Studies."[8] Numerous descriptions of species may also be found in Brumpt, in Lieske, and in Sartory. Certain species of economic importance will be described in the succeeding chapter.

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CHAPTER XI

BIOLOGIC ACTIVITIES OF THE ACTINOMYCETES

It is proposed to discuss in this chapter certain activities of the Actinomycetes which are of economic importance, namely their production of disease in man and animals, their relation to potato scab, and their activities in the soil.

The Actinomycoses.—It is difficult to discuss the actinomycoses because of certain confusions which have arisen with regard to the terminology of the diseases, due to changing conceptions of the etiology. At first actinomycosis was considered a specific disease due to a single organism, *Actinomyces bovis*, characterized by the presence within the suppurative lesions of granules of characteristic form. That two different species of Actinomyces, the anaerobic type of Israel and the aerobic type of Bostroem, contended for position as the true cause of this disease did not greatly complicate matters. But the later investigations of Lignieres and Spitz,[8] of Magrou,[11] and of Magnusson[10] have shown that both the lesions and the characteristic granules of actinomycosis may be produced by certain species of bacteria. We must therefore revise our clinical conception of actinomycosis to include a multiple etiology, in which Actinomycetes play a part in only a proportion of cases.

Similarly, when Vincent discovered the *Actinomyces madurae* in cases of Madura foot, that disease came to be looked upon as a distinct entity with a single specific causative organism. This conception had to be abandoned with the further discovery that in some cases of Madura foot the characteristic granules in the pus may be composed of other species of Actinomycetes, or indeed of higher fungi having septate mycelium.

To eliminate some of the confusion due to these changing conceptions, Pinoy,[16] followed by Chalmers and Archibald,[2] introduced the following nomenclature: All cases of infection accompanied by chronic suppuration and deformity, in which the causative organism appears in characteristic lobulated granules, are referred to as *mycetomae.* If these be due to Actinomycetes, they are called *actinomycoses;* if caused by higher fungi, they are known as *maduromycoses.* But in a considerable number of infections with Actinomycetes, as with the acidfast group, neither deformity nor characteristic lobulated granules occur. Such cases are frequently referred to in clinical literature as *streptotrichosis,* owing to Petruschky's differentiation of Actinomyces and Streptothrix on the basis of club formation.

It would seem that three well-defined types of infection occur in man and animals that are caused by species of Actinomycetes. The most common form may be referred to as the "lumpy jaw" type or actinomycosis proper; it is caused by *Actinomyces bovis* (Israel type). A second form, Madura foot, is most commonly caused by *Actinomyces madurae* (Vincent). The third type is caused by acidfast Actinomycetes, of which *Actinomyces asteroides* (Eppinger) is found most frequently in man, *Actinomyces farcinica* (Nocard) in cattle. If it is borne in mind that the "lumpy jaw" type of actinomycosis may be clinically reproduced by certain bacteria, and that Madura foot may be clinically reproduced by some higher fungi, such an arrangement will not lead to confusion in a discussion of the pathogenic Actinomycetes. A few rare cases of infection with Actinomycetes not well known will not fit into the above classification. These are mostly pulmonary infections with non-acidfast species which do not form granules.

Actinomycosis Proper.—The lumpy jaw type of Actinomycosis is a chronic, progressive disease characterized by purulo-granulomatous lesions which contain the parasite in the form of small granules having a radiating structure, with hyaline clubs at the periphery as was indicated in the preceding chapter.

One of the most remarkable facts about Actinomycosis is the volume of literature which has been written about it, and the small amount of definite information contained therein. In spite of the fact that the disease has been well known for half a century, and that probably more than a thousand papers have been published on it, we are still somewhat uncertain as to the mode of infection and transmission, as to whether the disease may be contracted by man from cattle, for instance, and still a little in doubt as to the nature of the causative organism, whether one or several species of Actinomycetes are concerned.

Sulphur Granules.—The diagnosis of actinomycosis is established by finding the organism in the pus in the form of very characteristic "sulphur granules" (in German, "drusen"). These vary considerably in size, the larger ones being distinctly visible to the naked eye. They have a radiating lobulated structure which is quite characteristic. Where much pus is available, they are best searched for by diluting the pus somewhat and straining through several layers of gauze; the larger granules will be caught on the gauze and may be removed for examination. With smaller amounts it is better to spread the diluted pus in a Petri dish and go over it with a hand lens. With still smaller amounts make a wet preparation with strong sodium hydroxide solution. Occasionally the granules may be encrusted with lime salts, in which case treatment with dilute acid is necessary to make their structure visible.

The granules vary in color, the smaller ones being rather translucent and indefinite in color, the larger ones yellowish or even brown.

The granules are best observed with the low-power microscope lens. The interior of the granules will not stand out sharply in unstained preparations, but the clubs at the periphery are quite refractile and will appear as irregular lines marking the borders of the lobules (Fig. 98). Such an examination merely proves the presence of a lobulated granule of the parasite. In human cases this will most probably, but not certainly, be an Actinomyces. In bovine cases the identity must be proved by further examination. This may be done by making stained smears. To do this the granules are crushed between two slides and then spread in a thin film which is stained by Gram's stain. The Gram-positive branched filaments may be found. In general the club structures are disintegrated by such a procedure, but occasionally they may be recognized in the stained smear; they take the counterstain.

As was pointed out in the preceding chapter, these clubs are formed by a secretion of material in the membrane of the terminal filaments of the fungus, and are not entirely specific, similar material being secreted by various other kinds of organisms. This has been especially established by Magrou [11] in observations of a Staphylococcus, the cause of a disease known as "botryomycosis" in horses. This is a chronic inflammatory disease of the subcutaneous tissues associated with the presence of certain granules of lobulated structure, which were formerly mistaken for a fungus named Botryomyces. But Magrou's investigations have established that these granules are in reality small colonies of *Staphylococcus aureus,* surrounded by an acid-staining sheath of material very similar to that formed in actinomycosis.

Some years ago Lignieres and Spitz [8] described a disease in cattle under the name "actinobacillosis" which bears a great similarity to actinomycosis in its clinical characters, but is due to a Gram-negative bacillus. In the pus this organism is gathered together in clusters surrounded by a lobulated sheath so that granules are formed very much like those found in actinomycosis. Inoculation of pure cultures of the bacillus into cattle reproduces the disease and the granules. The same organism has been isolated from cases in Europe and North America.

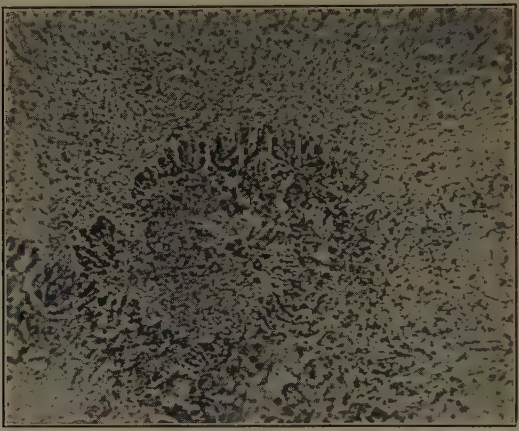

Fig. 98.—Photomicrograph of a crushed, unstained granule from actinomycotic pus. The dark irregular lines mark the position of the highly refractile clubs bordering the lobules of the granule.

Very recently Magnusson [10] has made an extensive investigation of actinomycosis in cattle and swine, and has come to the conclusion that a considerable number of the cases which have been in the past diagnosed as actinomycosis are not caused by Actinomycetes at all, but by bacteria of the type isolated by Lignieres and Spitz or Staphylococci of the type studied by Magrou. Only 41 per cent of the cases in cattle were due to Actinomycetes. Bovine "actinomycosis" of the jaw was in nearly all cases due to an Actinomyces, while the disease of the soft parts was in the majority of the cases an actinobacillosis, in the udder always a Staphylococcus infection. In swine the only cases observed were udder infections, of which two-thirds were due to Actinomycetes, the remainder to bacteria. Yet all of these cases would be considered as actinomycosis by their clinical characters and by the presence of radiating lobulated granules in the pus.

These findings explain, in part at least, the failure of most investigators to cultivate Actinomycetes from many of the cases, and indicate the importance of making a similar investigation in cases of human actinomycosis. There is already some evidence in the literature that bacteria play a part, if only a secondary one, in the etiology of human actinomycosis. Klinger found a small Gram-negative cocco-bacillus frequently associated with the ray fungus, which he named *B. actinomycetum-comitans.* This organism was found in 80 per cent of human cases of actinomycosis by Colebrook,[3] and has been isolated from a human case in this country by Bayne-Jones.[1] This organism is said to be quite different from the Actinobacillus of Lignieres and Spitz. These bacteria do not play any essential part in the pathogenesis of the disease, since in metastatic lesions the ray fungi may be found without any associating bacteria which can be demonstrated either in smears or cultures.

Actinomycetes have been reported as present in the crypts of the tonsils, and also in pus from pyorrhoea pockets and from dental root abscesses. Undoubtedly in the majority of cases at least, these have not been Actinomycetes, but peculiar granules composed of bacteria, as was first clearly demonstrated by Davis.[4] According to Davis they are composed of central threads of a filamentous bacterium to which fusiform bacilli are attached in parallel rows, forming a structure resembling a test-tube brush. These filaments form a tangled mass which results in the production of an irregular, brittle granule with a lobulated periphery. These granules bear an extraordinarily close resemblance to the granules of Actinomycetes, especially in stained sections, where the bacterial cells tend to fuse in amorphous masses at the periphery, thus resembling clubs. Such bacterial granules have also been observed in sputum, and the author recently found them in pus from an abdominal abscess. Very recently Tunnicliff and Jackson [20] have succeeded in cultivating these organisms anaerobically in the typical test-tube brush form. They have named the species *Vibriothrix tonsillaris.*

Taking into consideration all of the above facts, it will be seen that the *mere finding of radiating lobulated granules in pus is not sufficient evidence to establish a diagnosis of actinomycosis.* One must further prove that these granules are composed of fine branched filaments characteristic of the mycelium of the Actinomycetes.

Cultivation of Actinomycetes from Actinomycosis Proper.—Two distinct types of Actinomycetes have been cultivated from cases of actinomycosis of the "lumpy jaw" type in man and animals, one aerobic, the other anaerobic. The names *Actinomyces bovis* and *Actinomyces hominis* have been applied rather indiscriminately to both of these. It is now fairly clear that the aerobic species is a contaminant having nothing to do with the disease. Topley and Wilson [21] have recently proposed retaining the name *Actinomyces bovis* for the anaerobic species of Israel, and renaming the aerobic species of Bostroem *Actinomyces graminis,* which would help to avoid much confusion if adopted.

The Aerobic Species of Bostroem.—The aerobic type has only rarely been cultivated. Bostroem experienced great difficulty in obtaining a growth from most cases. For example, he found it necessary to inoculate large numbers of tubes of culture media to insure obtaining a growth in some, and states that out of seven hundred tubes inoculated with material from eleven different cases of bovine actinomycosis, only twelve showed a growth! Since it is now well known that saprophytic Actinomycetes are common air contaminants, Bostroem's results may well be questioned. It is quite possible that a careful bacteriologist would find twelve out of seven hundred tubes contaminated with Actinomycetes after inoculating them with sterile water. Experimental inoculations of both laboratory animals and of cattle with Bostroem's organism have failed to lead to infection. One may therefore seriously question the relationship of the aerobic form to the disease actinomycosis.

The finding of aerobic forms by Bostroem has been confirmed by only a few subsequent investigators. Nevertheless, this organism has been recognized as the cause of actinomycosis by many, and descriptions of it based upon Bostroem's original cultures have been handed down through the textbooks for a number of years.

The Actinomyces of Bostroem grows readily on all ordinary media. It forms a characteristic actinomyces growth, the mycelium usually yellow in older cultures, the aerial mycelium sulphur-yellow in color. No soluble pigment is formed. Gelatine is liquefied, milk is first coagulated and then peptonized. The mycelium does not readily fragment. Conidia are formed by the aerial mycelium in loose spirals.

The Anaerobic Species of Israel.—The anaerobic type of Israel has been found repeatedly by others, is pathogenic to laboratory animals and reproduces the disease in cattle. It therefore is probably the true etiologic agent in this disease. The same species has been isolated from cases in both man and cattle.

The Actinomyces of Israel is a species of the readily fragmenting type. Although in tissues mycelium predominates, in cultures the growth is more like that of bacteria than of Actinomycetes, mycelium being formed but scantily at the initiation of growth, fragmentation and post-fission movements leading to the development of numerous bacteria-like forms (Fig. 92).

The organism is not strictly anaerobic. It should perhaps be designated as microaerophilic. When first isolated in agar shake cultures, growth is best at a level one to two centimeters below the surface of the agar. After one or two subcultivations, some growth can be obtained under aerobic conditions, and occasionally growth will be obtained both aerobically and anaerobically in initial cultures. The growth on artificial media is always slow and rather scant. No growth occurs on potato, gelatine, and similar routine media. Most authors note that the addition of blood or blood serum is almost essential for first isolation. The author recently isolated a strain, however, which failed to grow on media containing blood or blood serum, which grew only on veal infusion agar. Deep colonies vary from lenticular to " fuzzy " types, depending on the degree of mycelium production. Surface colonies may be firm, mealy, or pasty, depending upon the same conditions. No aerial mycelium is formed, nor are there any evidences of conidia. No pigment is formed. It is very difficult to keep cultures alive.

Israel succeeded in isolating this organism by inoculating pus directly into hen's eggs under aseptic conditions. It has since been cultivated by a number of other bacteriologists. Wright[26] grew this organism from all of fifteen cases of both bovine and human actinomycosis. He was able to produce infection in laboratory animals with the production of typical granules with clubs. Clubs were also formed in first cultures in media containing serum. This type has recently been reisolated from a human case by Bayne-Jones,[1] his strain also forming clubs in media without serum. Magnusson [10] cultivated the Israel type of Actinomyces from fifty-four out of sixty-one cases of lumpy jaw in cattle, and tested the pathogenicity of these strains for various animals. In general the animal inoculations were negative save for cattle, eight out of thirty-two inoculations proving positive, with reproduction of the typical disease, and the formation of typical granules. In two cases positive results were obtained with swine.

It would seem, therefore, that the anaerobic type of Israel has a good claim to being considered the true cause of actinomycosis, having been isolated more regularly than others, and fulfilling Koch's postulates. It must be remembered, however, that numerous investigators have failed to cultivate any species of actinomyces from cases they have studied, and have failed to transmit the disease even by direct inoculations of pus from an infected animal to a healthy one. It would also appear from the work done so far that the human and bovine cases are caused by the same organism.

Actinomycosis in Lower Animals.—Actinomycosis occurs in a variety of animals, both wild and domesticated. Moody has described lesions, clearly actinomycotic, in the bones of a fossil rhinoceros. The disease is known to occur in deer and moose, sometimes in epidemic form. It has been noted in practically all of the domesticated animals, but mainly in the herbivorous species, and particularly in cattle. The disease in cattle occurs in all parts of the world, but is more frequent in certain areas. Thus in the United States many more cases are observed in the slaughter houses of the Middle West than in the eastern or southern states. It is annually a cause of great economic loss.

There is a great deal of evidence to indicate that the infection in cattle is contracted from contaminated hay, straw, or grain, and that the disease is not transmitted from animal to animal. As direct evidence of this, the finding of particles of such vegetable matter in the older lesions may be cited. A number of authors have reported the presence of particles of hay or grain, especially the awns of barley, covered with a growth of Actinomyces, in the tonsils, the soft tissues about the teeth or in the tongue of cattle and swine. Indirect evidence is given in the fact that the disease is more prevalent in cattle fed on dried vegetable matter than those on green feed, and occurs often in epizootic form after a dry season, or particularly after feeding hay which has been imperfectly cured. In Europe the disease appears to be more prevalent in moorlands or flooded areas. This may be due to the fact that hay is kept moist so that the organisms have a chance to grow upon it.

In cattle the disease occurs most frequently in the mouth parts, involving the tongue, the tissues about the teeth, the jaw bone, and in some cases the cervical lymph nodes. Lesions of the tongue are frequently referred to as " woody tongue " on account of the induration of the tissues.

According to Magnusson[10] " woody tongue " is never caused by the Actinomyces, but is always due to the Actinobacillus of Lignieres and Spitz. This is the most frequent form of the disease in cattle. It is particularly prone to be disseminated by way of the lymph vessels, resulting in abscesses in the submaxillary and cervical lymph glands.

Actinomycosis of the jaw bones, "lumpy jaw" proper, occurs through invasion of the tissues of the jaw bone by way of the peridental membrane. The organisms are probably introduced on bits of hard food material forced into the soft tissues about the teeth during chewing. They grow there, and gradually extend through the tissues between the tooth and the alveolar process, finally involving the jaw bone proper. The disease may be confined to the periosteum in some cases, but usually involves the bone marrow itself. The growth of the parasite gives rise to very characteristic tissue changes. In the immediate vicinity of the organisms the bony tissue is destroyed and an abscess is formed. But between these abscesses there is an overgrowth of bony tissue. As a result of these two processes there is formed a large swelling on the jaw bone, composed of new bone tissue which is honeycombed in all directions, so that it has a spongy texture.

The abscesses in the bone finally break through to the exterior either into the mouth or on the skin surface, forming fistulae which discharge pus.

Primary lesions of the lungs and of the intestinal tract also occur in cattle, but are not nearly so frequent as those of the mouth parts.

Actinomycosis in Man.—In man also there is both direct and indirect evidence that the disease is contracted from infected vegetable matter gaining access to the tissues. The direct evidence is not so great, though a small number of cases are on record where grains or bits of straw were demonstrated in the primary lesions. Indirect evidence is offered in statistical analyses of cases by occupation, and in careful analyses of case histories.

Actinomycosis in man is most frequent in adult males, and a very considerable proportion of the cases occur in men engaged in occupations which would expose them to the possibility of inhaling, ingesting, or otherwise having dried vegetable matter introduced into the tissues. Farmers, gardeners, stablemen, tanners, and people engaged in similar occupations comprise probably more than half the cases. It might be argued that people so engaged are also in contact with cattle and liable to infection from that source. But the disease is not particularly frequent among slaughter-house employees. On the other hand, it has been found to occur rather commonly in the men engaged in Italy in packing wines in straw. In many cases an inquiry into the history will indicate a source of infection which would not be suspected from the occupation. Thus Foulerton [5] reports several cases, that developed in city dwellers after a vacation on a farm. In quite a few cases of actinomycosis of the mouth parts, it is recorded that the individual was in the habit of chewing straws.

Geographic Distribution.—There is some tendency to a varied geographic distribution. Naturally the disease occurs more frequently in country districts than in the cities. It is found in all parts of the world. But in the United States it is clearly most frequent in the North Central states. The map shown in Fig. 99. is taken from an article by Sanford,[18] and represents an analysis of nearly seven hundred cases either reported in the literature or collected by questionnaires. Such a series of cases cannot be truly representative, because cases reported in the literature by physicians will be to a certain extent determined by the medical population of the district as well as by the prevalence of the disease. The high incidence in New York and Massachusetts is perhaps to be explained on this basis. But there is clearly evident a greater proportion of cases in Illinois, Wisconsin, Minneosta and North Dakota. This also corresponds with a greater incidence of the disease in cattle in this district.

Location of Lesions.—In man the disease has been known to involve all parts of the body, but occurs as a primary lesion most frequently in the neighborhood of the face and neck. Second in frequency are primary lesions of the abdominal cavity, and third the cases primary in the lungs.

Cervico-facial actinomycosis in man usually originates in the mouth parts, the mode of invasion probably being the same as in cattle. But the jaw bone is rarely involved. Instead the infection tends to spread through the soft parts. The infection is usually somewhat more acute than in bovines, suppuration being more pronounced, and overgrowth of granulation tissue less so.

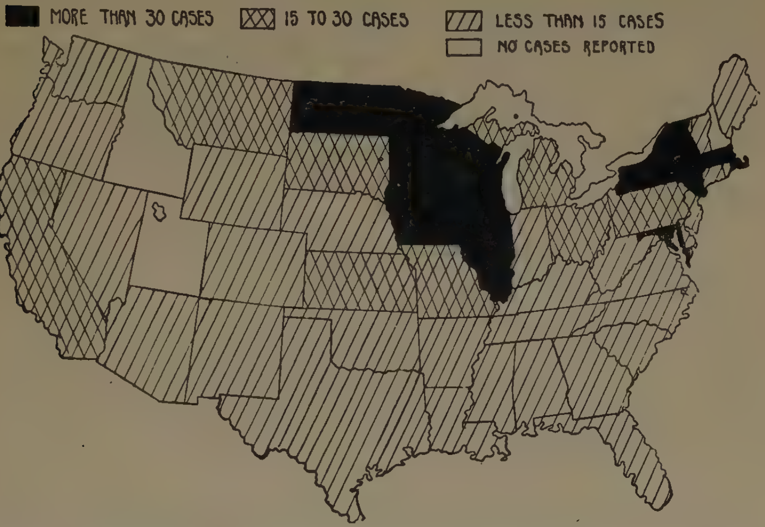

FIG. 99.—Map showing the distribution of human actinomycosis in the United States. After Sanford.

Abdominal actinomycosis occurs much more frequently in man than in cattle. A large proportion of cases apparently have their origin in the neighborhood of the appendix or cecum, and lead to the development of walled-off pericecal abscesses. Such cases are frequently operated upon for chronic appendicitis or appendiceal abscess, and show a pronounced tendency to drain pus for some time. A rather large number of cases primary in the internal female genital organs are on record. Primary abdominal lesions frequently result in secondary liver abscesses, and there are some cases of actinomycotic liver abscess in which no primary lesion in the abdomen can be demonstrated. Abdominal actinomycosis is possibly contracted by swallowing particles of infected vegetable matter which may lodge in the appendix.

Primary pulmonary actinomycosis may develop about particles of vegetable matter inhaled. At least one case is on record where an awn of barley was found in the center of a primary pulmonary lesion. In some cases lesions of the lung are secondary to lesions of the mouth parts. Lesions of the lungs vary in character from a subacute bronchopneumonia to a chronic disease resembling tuberculosis, with the development of cavities.

Not a few cases are primary in the subcutaneous tissues, resulting from wounds in most cases, though frequently this cannot be definitely established by the history. They are characterized by a chronic, progressive inflammatory reaction with the production of fistulae that discharge pus over a long period of time.

Any suppurative inflammatory reaction which stubbornly resists treatment, but tends to discharge continuously, should lead one to suspect the possibility of actinomycosis.

Madura Foot.—Madura foot is a chronic purulo-granulomatous infection of the feet occurring most frequently in tropical countries, but found occasionally in all parts of the world. Twenty or more cases have been reported from North America. Infection occurs through wounds of the feet, particularly those due to thorns or other vegetable matter. The tropical distribution is probably due to the custom of going barefoot. Like the other fungus infections, the disease is chronic and progressive, the disease frequently lasting over a period of years. The name is derived from that of a town in India from which the disease was first described. It is referred to with increasing frequency as mycetoma pedis.

Two types of the disease are known, one accompanied by white or yellowish granules in the pus, the other with black granules. The majority of the former cases are due to *Actinomyces madurae*, the majority of the latter are caused by Fungi Imperfecti of the genus Madurella. But it is now clear that the color of the granules is not a definite or constant character, and that a multiplicity of species of fungi are involved in the etiology. Following the nomenclature of Chalmers and Archibald,[2] these cases are now divided into two groups according to the nature of the causative organisms, actinomycoses and maduromycoses.

Maduromycoses of the foot have been caused by no less than seventeen species of Fungi Imperfecti, according to Gammel,[6] who has presented an excellent review of the subject. These are contained in the genera Madurella, Indiella, Glenospora, Scedosporium, Allescheria, Aspergillus, and Penicillium. But the majority of cases are due to species of Madurella.

Infections with species of Madurella are accompanied by the presence of black granules. These are firm and of considerable size. They are composed of densely packed, coarsely articulated filaments of mycelium. Large multicellular chalmydospores are also formed. These dense masses of dark mycelium are perhaps to be looked upon as sclerotia, which are definitely produced in artificial cultures. These are sterile sclerotia. No ascospores are formed, multiplication in cultures takes place by fragmentation of the mycelium. The position of the fungus is obscure, but it clearly belongs with the Dematiaceae of the Fungi Imperfecti. Six species have been differentiated on minor characters, of which *Madurella mycetomi* is the type.

The genus Indiella comprises organisms of a similar character which, however, produce white grains. They have never been cultivated.

Actinomycetes in Madura Foot.—Thirteen species of Actinomycetes have been recovered from cases of Madura foot according to Gammel.[6] Many of these, have, however, been poorly differentiated. The organism isolated by Vincent and named *Actinomyces madurae* is most frequently encountered.

The granules of *A. madurae* as examined in pus from a case of Madura foot have in general the same structure as the sulphur granules from the lumpy jaw type of actinomycosis, consisting of a central basic staining portion composed of a dense meshwork of the fine filaments and a peripheral zone composed of acid-staining clubs. But these bodies are much smaller and finer in structure than those in actinomycosis proper.

Actinomyces madurae may be readily cultivated on ordinary media, and is aerobic. The growth on solid media is usually somewhat mealy and wrinkled, generally creamy white in color, though at times cultures will suddenly develop a most brilliant crimson color. The aerial mycelium is very scant and white in color. There is no soluble pigment on any media. Starch is digested, milk peptonized, and gelatine liquefied. The mycelium readily undergoes fragmentation in older cultures. No spirals are formed in the aerial mycelium.

Gammel obtained reports of twenty-four cases of mycetoma which have occurred in the United States. Of these eighteen were actinomycoses.

Madura foot may occasionally, though very rarely, extend up the leg by way of the lymphatics, but does not tend to metastasize to other parts of the body and endanger life. On the other hand, it tends to progress steadily and does not respond to any sort of medication. The best treatment is amputation.

Acidfast Actinomycetes.—The acidfast Actinomycetes form a group which is not of very great practical importance, because infections caused by them are rare, but of great scientific interest since they form a distinct connecting link between the bacteria proper and the higher fungi.

A number of apparently different species of acidfast Actinomycetes have been isolated from both domestic animals and man. They all bear a rather close resemblance to each other in their morphological, cultural, and pathogenic properties, tending to form mealy growths on culture media, with readily fragmenting mycelium, and forming pseudotubercles in the tissues.

The acidfastness of these forms is not so pronounced as that of the tubercle bacillus, i.e., they may be decolorized with acids in a shorter period of time; but it is distinct enough to clearly demonstrate the organisms in tissues or exudates. In general they are much more definitely acidfast in tissues and exudates than in cultures, tending to lose this property after continued cultivation.

They may be distinguished from the other pathogenic Actinomycetes by the readiness with which they may be cultivated on artificial media, their cultural characters, and particularly by their high and constant virulence for laboratory animals. Their relationship to the acidfast bacteria is indicated not only by their acidfastness. Several species, notably *A. asteriodes*, form a wrinkled mealy growth of yellowish-orange color which bears a very close resemblance to that of the tubercle bacillus on solid media. The lesions of the natural infection may also closely simulate tuberculosis; the experimental infections are generally more acute than is tuberculosis, producing more of a suppurative reaction and necrosis, with less granulomatous reaction. But the close relationship is most clearly indicated by the immunological reactions. Thus, Nelson and Henrici [15] found that with complement fixation reactions the acidfast Actinomycetes showed a closer relationship to the acidfast bacteria than they did to the non-acidfast Actinomycetes.

Three species have been obtained from infections in animals; *A. farcinica* from cattle, *A. caprae* from goats, and *A. canis* from dogs.

A. farcinica produces a disease of cattle characterized by a spreading subcutaneous lymphangitis with localized abscesses, somewhat resembling glanders in horses, and designated "farcin du boeuf" by Nocard, who first described it and isolated the organism. It occurs in France and on the island of Guadeloupe in the French West Indies, but has also been reported from other parts of the world. The organism forms a pale yellow wrinkled growth on solid media, with a powdery aerial mycelium in older cultures. No change occurs in milk or gelatine. The organism is pathogenic for guinea pigs, cattle and sheep, not for other animals.

A. caprae, described by Silberschmidt, is very similar to the above, differing in forming a more whitish growth on solid media, a more pronounced fragmentation of the mycelium, and in forming a pellicle of rosy color on milk. It is pathogenic for both rabbits and guinea pigs.

Several strains have been isolated from spontaneous infections in dogs which have been designated *A. canis,* though none have been adequately described. One studied by Musgrave and Clegg appeared to be identical with *A. caprae.*

One of the varieties which have been found in man, *Actinomyces asteroides,* is the best known. This species was discovered by Eppinger and named by him *Cladothrix asteroides.* The same organism has been found in a number of other cases since. On solid media it forms a wrinkled growth of mealy consistency varying from pale yellow to deep orange in color, depending upon the age of the culture and the composition of the medium. Aerial mycelium is rarely formed; when present it is white and very scant. It does not liquefy gelatine nor peptonize milk. The organism is very pathogenic to guinea pigs and rabbits.

An acidfast strain isolated from a human case by Ayoyama and Myamoto resembled *A. caprae* in color. Another one isolated by Birt and Leishman gave a snow-white growth and peptonized milk. A fourth type obtained by Berestneff liquefied gelatine and was not pathogenic for laboratory animals.

Henrici and Gardner [7] isolated an acidfast Actinomyces from a human case which resembled *A. asteroides* in forming a buff-colored mycelium, but with a very pronounced production of aerial mycelium, so that the growth was of a pronounced chalky white; and which differed from the other acidfast species in being actively proteolytic, liquefying gelatine, producing in milk first a rennin coagulation and then peptonization, and giving a pronounced dark brown "tyrosinase reaction." But as was mentioned in the preceding chapter, these characters have not remained constant, save the white aerial mycelium and the darkening of protein-containing media. They named their organism *A. gypsoides*. It was very pathogenic for rabbits and guinea pigs.

In 1920 Henrici and Gardner [7] were able to collect from the literature records of 26 cases in man. In all but three of these the infection was primary in the lungs or peribronchial lymph nodes. In the lungs there is produced a caseous bronchopneumonia with eventually softening and cavity formation, but there is a pronounced tendency for the organisms to become distributed through the blood with the formation of abscesses in other viscera, especially the brain. A rather large proportion of the reported cases have died from brain abscesses, and in some of these the pulmonary lesions were not discovered until after death. There were two cases of primary peritonitis following a simple surgical exploration of the abdomen.

Infections with the acidfast actinomycetes are to be differentiated from tuberculosis. In pronounced pulmonary cases this is not easily done, and undoubtedly these infections have been mistakenly diagnosed as tuberculosis a number of times. In the sputum the organism readily undergoes fragmentation, and being acidfast the fragments resemble tubercle bacilli very closely. They are more variable in length, and sooner or later long branched filaments will be found.

Although these forms grow readily on ordinary culture media, they grow more slowly than bacteria, and isolation from sputum by plating is difficult. But by guinea pig inoculations pure cultures may be readily obtained. This would seem to be the procedure of choice for establishing the diagnosis. If inoculated intraperitoneally the animals generally die in less than a week, with very characteristic miliary white nodules over the peritoneal surfaces, especially in the omentum. The cultures retain their virulence for surprisingly long periods. Thus Musgrave and Clegg found subcultures of Eppinger's strain of *A. asteroides* fully virulent after twenty years.

Soil Actinomycetes.—It is a remarkable fact that, though relatively small numbers of pathogenic species of Actinomycetes have been fairly well known for a number of years, the great abundance and importance of saprophytic forms in the soil has received general recognition only very recently, particularly through the work of Waksman.[23, 24]

Actinomycetes form about 17 per cent on the average of all the microorganisms which may be cultivated from soil; the proportion going much higher, over 40 per cent, in some samples. They are more numerous in soils containing an abundance of organic matter than in poorer soils. The reaction of the soil seems to be the principal limiting factor, growth being inhibited by even slight amounts of acidity. They are, therefore, practically absent in sour waterlogged soils, as in bogs and peat. They are actually most numerous near the surface, but may extend to a greater depth than the bacteria, so that deeper in the soil they form a larger proportion of the total microbic population.

They do not fix atmospheric nitrogen, nor do they convert ammonia to nitrates. On the contrary they reduce nitrates to nitrites, in which form nitrogen is apparently assimilated by them. But the reduction is not carried on to the formation of ammonia or nitrogen. They rapidly break down proteins to simpler compounds, so that directly or indirectly they are active in ammonification.

The soil Actinomycetes are perhaps more important in their activity in decomposing the more complex carbohydrates, since practically all species break up starch, and many of them cellulose. Thus they are well adapted to initiate the decomposition of dead plant matter, since they can attack the three most complex and abundant of the organic compounds present: protein, starch and cellulose. The very slow and imperfect decomposition of the plant matter in acid bog soils may be due, in part at least, to the absence of Actinomycetes.

Potato Scab.—Potato scab is a disease of the tubers characterized by the production of warty excrescences or "scabs" on the surface, which eventually break down and slough off. It is the most common disease of the potato. Specimens showing this disease may be found in almost any sack of potatoes. It is a source of considerable economic loss, since scabby potatoes do not command so high a market price, because in preparing the potatoes for food a considerable amount is wasted in removing the scabs, but mainly because the plants infected do not give so high a yield as healthy ones. It is not easily controlled. There are several other diseases of potatoes due to higher fungi which resemble ordinary potato scab, but that caused by Actinomycetes is the most common.

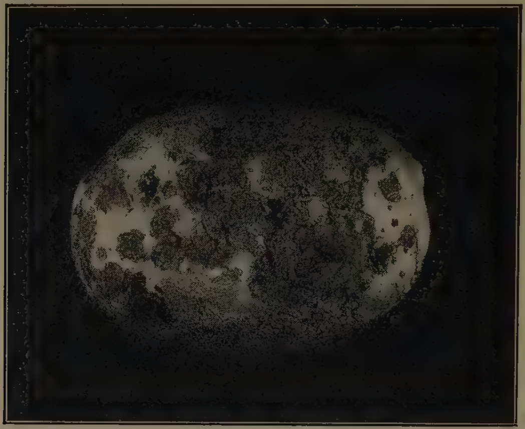

FIG. 100.—Potato scab.

The effect of the parasite is mainly irritation at the point of invasion, leading to an overgrowth of cork cells in the epithelium, forming the warty excrescence. The outer layers of corky material break down and slough off, forming what might be considered "ulcers." The parenchymal cells beneath the scab lose their starch and develop oil globules, but there is no extensive disintegration of the potato proper. However, the scab lesions provide a break in the protective coat of the potato so that other fungi may invade. Although the disease develops only in the growing tubers, not in the stored potatoes, scabby potatoes during storage are more liable to rots than are normal ones.

The disease also occurs to a lesser extent on beets and other root crops.

The organism was first described by Thaxter, and named *Oöspora scabies.* But Lutman and Cunningham [9] made an extensive investigation of the disease and showed that the parasite is an Actinomyces. The organism which they cultivated gave a dark brown color in protein-containing media, a characteristic of the soil species which Gasperini had named *Actinomyces chromogenus.* But as was pointed out in the preceding chapter, this reaction is common to a large series of Actinomycetes now referred to as the Chromogenus group. For a time therefore there was recognized a distinct species in the group known as *Actinomyces scabies,* which, however, could only be identified by inoculating growing potatoes under aseptic precautions.

But with the development of better methods of identifying Actinomycetes, particularly the use of synthetic media by Krainsky and Waksman, means were provided for more closely determining the specific characters of the scab organism. Such an investigation has been made by Millard and Burr,[13] who have come to the conclusion that there is no specific species responsible for the disease of potatoes, but that a large number of varieties may be involved. They obtained eighteen strains from scabby potatoes, which for the most part proved to be different species; in some cases two different species were obtained from the same lesions. Eleven out of twenty-four varieties produced scab in carefully controlled inoculation experiments, though some of these species were obtained from soil or grass, and not from scabby potatoes. There is, therefore, no valid reason for continuing to recognize a specific "*Actinomyces scabies,*" although Millard and Burr retain this name for one of the species isolated by them which seems to fit Thaxter's original description. The various species isolated by these authors have been carefully described by them and named.

Since such a wide variety of soil Actinomycetes can produce scab, it would seem that these organisms must be present in all soils and that it would be hopeless to attempt to control the disease. But experience proves that this is not the case. Though scab will develop in new soil, it becomes progressively worse if the soil is used continuously for the cultivation of potatoes, and is always much more extensive if scabby potatoes are used for seed than if scab-free seed potatoes are selected. It would appear, therefore, that although there is a multiplicity of species potentially capable of producing scab, only a few of these actually do develop the power of invading the tubers.

The occurrence of potato scab is considerably affected by soil and climatic conditions. High temperatures and excessive moisture favor the development of the disease. But the most important factor is the reaction of the soil. Like the other soil Actinomycetes, the scab organisms are sensitive to acid. It has been shown that, if the soil reaction is maintained at pH 4.8 or lower, scab will not develop.[22] This may be accomplished by adding sulphur to the soil,[12] which becomes oxidized to form sulphuric acid, or by using ammonium sulphate as a fertilizer, which makes the soil more acid since the growing plants utilize the ammonium radicle but not the $—SO_4$. Liming the soil tends to increase scab, by making the soil more alkaline.

Other important control measures are crop rotation, selection of seed, and disinfection of the seed potatoes. The latter is accomplished by immersing the potatoes in a weak solution of bichloride of mercury.

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